National Library
of Canada

Acquisitions and
Bibliographic Services Branch

395 Wellington Street
Ottawa, Ontario
K1A 0N4

Bibliothèque nationale
du Canada

Direction des acquisitions et
des services bibliographiques

395, rue Wellington
Ottawa (Ontario)
K1A 0N4

Your file Votre référence

Our file Notre référence

NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Reproduction in full or in part of this microform is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30, and subsequent amendments.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

La reproduction, même partielle, de cette microforme est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30, et ses amendements subséquents.

Canada

UNIVERSITY OF ALBERTA

CLONING AND EXPRESSION OF A TRUNCATED T CELL RECEPTOR α mRNA FROM MURINE KIDNEY

BY

©

JOAQUIN MADRENAS

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of **DOCTOR OF PHILOSOPHY**

IN

MEDICAL SCIENCES (IMMUNOLOGY)

EDMONTON, ALBERTA

FALL, 1992

National Library of Canada
Acquisitions and Bibliographic Services Branch
395 Wellington Street
Ottawa, Ontario
K1A 0N4

Bibliothèque nationale du Canada
Direction des acquisitions et des services bibliographiques
395, rue Wellington
Ottawa (Ontario)
K1A 0N4

Your file Votre référence

Our file Notre référence

The author has granted an irrevocable non-exclusive licence allowing the National Library of Canada to reproduce, loan, distribute or sell copies of his/her thesis by any means and in any form or format, making this thesis available to interested persons.

L'auteur a accordé une licence irrévocable et non exclusive permettant à la Bibliothèque nationale du Canada de reproduire, prêter, distribuer ou vendre des copies de sa thèse de quelque manière et sous quelque forme que ce soit pour mettre des exemplaires de cette thèse à la disposition des personnes intéressées.

The author retains ownership of the copyright in his/her thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without his/her permission.

L'auteur conserve la propriété du droit d'auteur qui protège sa thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

ISBN 0-315-77365-0

Canada

THE JOURNAL OF IMMUNOLOGY
9650 ROCKVILLE PIKE
BETHESDA, MD 20814-3994
Phone: 301-530-7197
FAX: 301-571-1813

May 28, 1992

Dr. Joaquin Madrenas
Transplend Lab. - CRC Bldg.
8249 - 114 St.
Edmonton, Alberta
CANADA T6G 2R8

Dear Dr. Madrenas:

Thank you for your letter requesting permission to use material from the article entitled: "Thymus-Independent Expression of a Truncated T Cell Receptor-Alpha mRNA in Murine Kidney", by Madrenas et al., published in THE JOURNAL OF IMMUNOLOGY 148:612-619, 1992.

Permission is granted for use of the data/information you specified from the above, provided that THE JOURNAL OF IMMUNOLOGY is credited as the source.

Sincerely yours,

Joseph F. Saunders

Joseph F. Saunders, Ph.D.
Executive Director and
Managing Editor
THE JOURNAL OF IMMUNOLOGY

JFS:df

UNIVERSITY OF ALBERTA

RELEASE FORM

NAME OF AUTHOR: **JOAQUIN MADRENAS**

TITLE OF THESIS: **CLONING AND EXPRESSION OF A TRUNCATED T CELL RECEPTOR α mRNA FROM MURINE KIDNEY**

DEGREE: **DOCTOR OF PHILOSOPHY**

YEAR THIS DEGREE GRANTED: **1992**

Permission is hereby granted to the University of Alberta Library to reproduce single copies of this thesis and to lend or sell such copies for private, scholarly or scientific research purposes only.

The author reserves all other publication and other rights in association with the copyright in the thesis, and except as hereinbefore provided neither the thesis nor any substantial portion thereof may be printed or otherwise reproduced in any material form without the author's prior written permission.

Joaquin Madrenas

c/ Vic, 12, pral. 2ª

BARCELONA - 08006

SPAIN

Date: July 17, 1992

UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **CLONING AND EXPRESSION OF A TRUNCATED T CELL RECEPTOR α mRNA FROM MURINE KIDNEY** submitted by **JOAQUIN MADRENAS** in partial fulfillment of the requirements for the degree of **DOCTOR OF PHILOSOPHY** in **MEDICAL SCIENCES (IMMUNOLOGY)**.

Dr. P.F. Halloran, Supervisor

Dr. R.C. Bleackley

Dr. B. Singh (Dr. K. Kane)

Dr. L. Guilbert

Alex Rabinovitch

Dr. A. Rabinovitch

Dr. R.P. Sekaly
External Examiner, McGill University

Date: July 17, 1992

To Teresa, for everything that her love makes possible.

To Oriol R. and Clara R., because they deserve the best and..........

hoping they will find this thesis worth reading.

ABSTRACT

During studies on gene expression in the kidney, we observed that murine kidney expresses a truncated form of TCR α mRNA. This transcript was not associated with the presence of complete TCR α mRNA or detectable TCR β or δ transcripts, indicating that the truncated TCR α mRNA could not be attributed to blood contamination of the kidney RNA preparation. The truncated TCR α message contained the Cα region, as indicated by hybridization with an intra-Cα oligoprobe, by amplification of the Cα region with the polymerase chain reaction (PCR) from total kidney mRNA, by sequencing of, and hybridization with, the amplified products, and by RNase protection assay with a TCR α C region probe. The transcript was expressed in interstitial cells, predominantly located in the medulla. The truncated TCR α mRNA was detected in a renal cell fraction containing large cells in which no expression of CD3, Thy-1, or NK-1.1 was detected, implying that these cells are not mature T cells, do not express a functional TCR, and are not NK cells. The cells expressing the truncated TCR α mRNA were radiosensitive, bone marrow dependent, and were not thymus-dependent. The transcript was not detected in bone marrow. Expression of the truncated TCR α mRNA was not dependent on an intact recombinase activity.

The truncated TCR α mRNA is normally processed as it is correctly spliced at the Cα region, with conservation of the transmembrane domain, is polyadenylated, and can be detected in the cytoplasm. Immunoprecipitation studies are consistent with the hypothesis that the truncated TCR α mRNA is

translated into a ~30 kd protein.

In vivo administration of cycloheximide is associated with slight decrease of the truncated TCR α mRNA, and with the appearance of a larger band (1.7 kb) reacting with a TCR α cDNA probe. The size of this band is compatible with the size of the complete message for TCR α. No evidence of renal T cell infiltration was obtained after cycloheximide administration.

These results show that murine kidney contains a population of radiosensitive, bone marrow-derived, and thymus-independent large interstitial cells which express a truncated TCR α mRNA. These cells may have attempted TCR α gene rearrangement, suggesting that they may be related to the lymphoid lineage. In these cells, the truncated TCR α mRNA may play a role in controlling gene rearrangement through the transmembrane expression of a truncated TCR α protein.

ACKNOWLEDGMENT

Many are the people and their circumstances that, consciously or unconsciously, have been of great help in the development of the ideas that are presented in this thesis. With the imposed space constraints, I would like to mention all of them.

I want to thank Dr. P.F. Halloran. He has been my mentor driving my mind through the exciting pathways of molecular immunology, and, sometimes, driving me nuts. However, our frequent sparking Wednesday discussions have often lead to 'hot' ideas, and particularly, to my admiration and respect for him.

I would like to thank Drs. R.C. Bleackley and B. Singh, members of my supervisory committee. They have taught me how to frame my thoughts scientifically in a true friendly environment of academic excellence.

I would also like to acknowledge the support of Drs. N.A. Parfrey and A. Rabinovitch, external examiners along my Ph.D. program, and Drs. J.F. Elliott and J. Kriangkum, for their collaboration and ideas in the development of anchored PCR in this particular project.

A very special acknowledgement goes to scientists, fellows, technologists, and students in the Transplantation Laboratory of the Division of Nephrology and Immunology at the University of Alberta. They have played a critical role in this project, providing the support, the help, and the right environment to 'be wild'. I, therefore, want to thank C. Baergen, P.G. Blake, A.P. Broski, S.M. Cockfield, A. Drake, J. Enns, N. Gill, H.R. Moody, F. Pazderka, V. Ramassar, J.R. Urmson, and D.H. Vincent.

This thesis has been possible through funding from the Medical Research Council of Canada, the Alberta Heritage Foundation for Medical Research, and the Kidney Foundation of Canada.

Parts of this thesis have been published. Madrenas, J., F. Pazderka, N.A. Parfrey, and P.F. Halloran. J. Immunol. 1992; 148: 612-619.

TABLE OF CONTENTS

ABSTRACT

ACKNOWLEDGMENT

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

CHAPTER ONE: CONTROL OF T CELL RECEPTOR GENE EXPRESSION . . 1

- INTRODUCTION 2
- STRUCTURE OF THE TCR COMPLEX 3
- MOLECULAR GENETICS OF THE TCR GENES 7
- REGULATION OF TCR GENE EXPRESSION 11
- CONTROL OF TCR GENE REARRANGEMENT 11
- CIS-ACTING REGULATORY ELEMENTS OF TCR LOCI AND THEIR TRANS-ACTING TRANSCRIPTION FACTORS 19
  - Proximal Regulatory Elements 20

Enhancer Elements 24

CONTROL OF TCR MONO-SPECIFICITY IN EACH T CELL: ALLELIC EXCLUSION 36

POST-TRANSCRIPTIONAL REGULATION OF TCR GENE EXPRESSION 40

OBJECTIVE OF THIS THESIS 42

CHAPTER TWO: MATERIAL AND METHODS 44

MICE 45

RENAL CELL FRACTIONATION 45

RNA EXTRACTION AND NORTHERN BLOTTING 46

POLYMERASE CHAIN REACTION (PCR) 49

SEQUENCING 51

MONOCLONAL ANTIBODIES 52

IRRADIATION AND BONE MARROW RECONSTITUTION EXPERIMENTS 53

IMMUNOPEROXIDASE STAINING 53

IN SITU HYBRIDIZATION 53

RNASE PROTECTION ASSAY 54

IMMUNOPRECIPITATION 55

CYCLOHEXIMIDE 57

CHAPTER THREE: RESULTS 58
DETECTION OF A TRUNCATED TCR α mRNA IN MURINE KIDNEY 59
THE CELLS EXPRESSING THE TRUNCATED TCR α mRNA ARE NOT T CELLS OR NK CELLS 62
THE TRUNCATED TCR α mRNA IS EXPRESSED BY RADIOSENSITIVE CELLS OF BONE MARROW ORIGIN IN A THYMUS-INDEPENDENT FASHION 67
THE EXPRESSION OF THE TRUNCATED TCR α mRNA DOES NOT REQUIRE AN INTACT RECOMBINASE ACTIVITY 73
THE TRUNCATED TCR α mRNA CONTAINS Cα 76
THE TRUNCATED TCR α mRNA CONTAINS A CORRECTLY SPLICED TCR α C REGION 79
THE TRUNCATED TCR α mRNA IS POLYADENYLATED AND CAN BE DETECTED IN THE CYTOPLASMIC FRACTION 84
DETECTION OF A TRUNCATED TCR α PROTEIN IN NORMAL KIDNEY 84
THE EFFECT OF CYCLOHEXIMIDE ON THE EXPRESSION OF TRUNCATED TCR α mRNA IN THE KIDNEY 87

CHAPTER FOUR: DISCUSSION 91
SUMMARY OF RESULTS 92
THE TRUNCATED TCR α mRNA AS AN INDICATOR OF A

PARTICULAR RENAL INTERSTITIAL CELL POPULATION . . . 94

POSSIBLE FUNCTIONS OF THE TRUNCATED TCR α mRNA AND/OR PRODUCT 102

A HYPOTHESIS FOR THE FUNCTION OF THE TRUNCATED TCR α mRNA EXPRESSED IN MOUSE KIDNEY 110

FUTURE PERSPECTIVES 111

CHAPTER FIVE: CONCLUSIONS 113

BIBLIOGRAPHY 116

APPENDIX I: CHARACTERIZATION OF THE 5' END OF THE TRUNCATED TCR α mRNA 152

LIST OF TABLES

Table I: Phenotype of the different renal interstitial cell fractions 68

LIST OF FIGURES

Figure 1.- Genomic organization of the murine TCR genes 8

Figure 2.- Upstream proximal cis-regulatory sequences of a murine Vβ gene. 21

Figure 3.- Organization of the human TCR α enhancer and the putative transcription factors binding to the different enhancer elements 26

Figure 4.- Cells isolated in each fraction as percentage of total number of cells isolated from each kidney. 47

Figure 5.- Location of the synthetic oligonucleotides used in these experiments in relation to the Cα gene segment 50

Figure 6.– Tissue distribution of complete (1.7 kb) and truncated (1.3–1.4 kb) TCR α mRNA 60

Figure 7.– Titration of hybridization for the TCR α and TCR β probes. 61

Figure 8.– In situ hybridization of kidney sections for TCR Cα 63

Figure 9.– The cells expressing the truncated TCR α mRNA are more abundant in the renal medulla 64

Figure 10.- The truncated form of TCR α mRNA is expressed by cells contained in Percoll fraction <40% 66

Figure 11- Effect of radiation on the expression of truncated TCR α mRNA by renal interstitial cells 69

Figure 12.- The renal cells expressing the truncated TCR α mRNA are bone marrow-dependent 71

Figure 13.- The truncated TCR α mRNA is not detected in the bone marrow. 72

Figure 14.- The population expressing the truncated form of TCR α mRNA is thymus-independent 74

Figure 15.- The expression of the truncated TCR α mRNA is not affected by the SCID mutation 75

Figure 16.- Northern blot of total RNA from different organs of normal mice probed with a synthetic oligonucleotide complementary to the TCR α C region 77

Figure 17.- Amplification of TCR Cα mRNA by PCR 78

Figure 18.- Sequence of the products obtained after PCR amplification of kidney total RNA with specific TCR α C region oligoprimers 80

Figure 19.- RNase protection assay of total kidney and spleen RNA from normal, nude, and scid mice. 81

Figure 20.- Two potential secondary structures can be formed in the TCR α C region 83

Figure 21.- The truncated TCR α mRNA is polyadenylated 85

Figure 22.- The truncated TCR α mRNA can be detected in the cytoplasm 86

Figure 23.- Immunoprecipitation of complete and truncated TCR α polypeptides with mAb anti-TCR α 88

Figure 24.- Effects of in vivo administration of cycloheximide on the expression of truncated TCR α mRNA in normal mouse kidney 89

Figure 25.- Sequence of the 5' end of the truncated TCR α mRNA 158

LIST OF ABBREVIATIONS

aa:	Aminoacid
Ag:	Antigen
bp:	Base Pair
C:	Constant Region
CD:	Cluster of Differentiation
cDNA:	Complementary Deoxyribonucleic Acid
CDR:	Complementarity Determining Region
D:	Diversity Region
DNA:	Deoxyribonucleic Acid
FITC:	Fluorescein Isothiocyanate
H:	Immunoglobulin Heavy Chain
Ig:	Immunoglobulin
J:	Joining Region
HBSS:	Hank's Balanced Salt Solution
IL:	Interleukin
LGL:	Large Granular Lymphocyte
MHC:	Major Histocompatibility Complex
mAb:	Monoclonal Antibody
mRNA:	Messenger Ribonucleic Acid
NK:	Natural Killer
nt:	Nucleotide
PBS:	Phosphate Buffered Saline

PCR:	Polymerase Chain Reaction
PMA:	Phorbol 12-myristate 13-acetate
SD:	Standard Deviation
SDS:	Sodium Dodecyl Sulphate
SSC:	Sodium Chloride - Sodium Citrate
TCR:	T Cell Receptor for antigen
V:	Variable Region
Zn:	Zinc

CHAPTER ONE:

CONTROL OF T CELL RECEPTOR GENE EXPRESSION

INTRODUCTION

Antigen (Ag) recognition by lymphocytes is the triggering event of the adaptive immune response. This event should be extremely specific in order to assure the proper function of the immune system in self-non-self discrimination. B and T lymphocytes have developed different structures to recognize Ag (reviewed in (1-8)). B cells recognize Ag in its soluble, non-processed form. Therefore, the B cell receptor for Ag, a membrane bound immunoglobulin (Ig) associated to several other molecules, is conformation-specific. In contrast, T cells can only recognize antigenic peptides derived from processing of the original Ag, and only when they are presented in the context of self-major histocompatibility complex (MHC) molecules. MHC restriction of the T cell repertoire is a unique feature of Ag recognition by T cells and defines two major populations of these cells: $CD4^+$ T cells, which recognize Ag in the context of class II MHC molecules, and $CD8^+$ T cells that recognize Ag when presented in the context of class I MHC molecules. Despite this major functional difference in the Ag recognition process, the structures involved on B cells and T cells are remarkably similar, as indicated by the analogies in the organization and regulation of the genes coding for these complexes, the use of similar structural patterns by the molecules forming these receptors, and the structure-function correlation in the receptor complexes (3, 9).

While the chemical structure of the Ig was known since the early 60s, the nature of the T cell receptor for Ag (TCR) was only established in the early 80s. The delay in the characterization of the TCR was due, in part, to the false assumption that T cells were using the antibody (Ab) gene pool to encode their Ag recognition structure (10). It was necessary to wait for the development of clonotypic monoclonal Ab (mAb) against T cells (11-13) and, particularly, for the development and application of subtractive hybridization (14, 15) to finally characterize the TCR at the protein and gene levels.

The discovery of the TCR genes and polypeptides is one of the hallmarks of the recent history of immunology because it has allowed for the experimental approach to multiple areas of interest in the field, ranging from the study of T cell ontogeny (16, 17) to the understanding of the development of tolerance (18).

STRUCTURE OF THE TCR COMPLEX

The TCR is a transmembrane, 80-90 kd disulfide-linked heterodimer formed by either the $\alpha\beta$ chains or the $\gamma\delta$ chains (reviewed in (1, 2, 19). The α chain of the TCR is an acidic glycoprotein of a maximum relative mass of 28 kd in mouse and 32 kd in man, in its deglycosylated form, and of 45 to 60 kd, in mouse and man, after addition of several N-linked oligosaccharides of the complex type. The TCR β chain is more basic than the α chain and has a relative mass, in deglycosylated form, of 32 kd in mouse and human, and of 40-55kd in glycosylated form. The TCR β chain contains both complex N-linked oligosaccharides and high mannose glycans. The γ chain is a 45-60 kd chain (30 kd in deglycosylated form). The δ chain is 40-60 kd (31 kd or 37 kd, in deglycosylated form, in mouse and in man respectively). Both γ and δ chains undergo N-linked glycosylation.

The polypeptides of the TCR heterodimer have an Ig-like arrangement with an external variable (V) region, of 102 to 119 aminoacids (aa), linked to a 87-113 aa constant (C) region by a diversity (D) region (in the case of β and δ chains) and a joining (J) region. The C region is connected to a 20-24 aa, hydrophobic transmembrane region, and a short (5 to 12 residues) cytoplasmic region (3). The four chains may contain, at least, two internal disulfide bonds, one in the V region and another one in the C region, which are compatible with a conformation in an Ig-like domain. In addition, the disulfide bond between the two chains forming the TCR is established between the proximal part of the C regions of the TCR polypeptides. The transmebrane region of the four chains has a net positive charge which may play a role in the interaction of the TCR heterodimers with CD3 molecules (252, 253).

By analogy to the Ig structure, it is assumed that the V region of the TCR polypeptides contains at least three hypervariable regions, also known as complementarity-determining regions (CDRs) (3). From a functional point of view, the V domains contain two hypervariable regions equivalent to the Ig CDRs, CDR1 and CDR2, and a third small loop called, sometimes, CDR4. The V-J junction in the TCR α and TCR γ, or the V-D-J junction in the TCR β and TCR δ form the CDR3 (3, 20). The currently most accepted model of MHC-restricted Ag recognition by the TCR assumes that the CDR3 contacts the Ag peptide directly, and it is in this region where the peptide specificity of the TCR is determined (3, 21, 254, 255). Confirmation of this model is awaiting co-crystallization of a TCR molecule engaging an MHC-peptide molecule.

The chains forming the characteristic TCR heterodimer ($\alpha\beta$ or $\gamma\delta$) have a preferential association with each other over other possible combinations (22). However, in addition to the two main TCR heterodimers ($\alpha\beta$ and $\gamma\delta$), three alternative types of TCR have been reported. First, two different forms of non-disulfide-linked $\gamma\delta$ heterodimers have been reported in human PBL (23-25). In contrast to the disulfide-linked form which uses a Cγ region encoded by the Cγ1 gene, these heterodimers use the Cγ region encoded by the Cγ2 (see below) (26). Second, a disulfide-linked $\gamma\gamma$ TCR has been shown in some human T cell clones (27, 28). This homodimer seems to be fully active as indicated by the ability of monoclonal antibody (mAb) anti-CD3 to stimulate interleukin-2 (IL-2) production by these clones. Third, a $\beta\delta$ TCR has been described in certain T cell leukaemia cell lines but it does not seem to be significant in the composition of the repertoire of normal T lymphocytes (29).

On the cell surface, the TCR is expressed in non-covalent association with at least five different species of CD3 molecules: the glycoproteins CD3γ and CD3δ, a non-glycosylated CD3ϵ molecule, and either the CD3ζ homodimer or the CD3ηCD3ζ heterodimer (2, 17). The long cytoplasmic domains of the CD3 molecules in comparison to the short corresponding domains of the TCR chains suggest that the CD3 molecules are involved in signal transduction after TCR-mediated T cell activation.

The co-expression of CD3 seems to be required for expression of a functional TCR heterodimer on mature T cells (2). However, in immature thymocytes, the TCR β chain can be expressed on the cell surface, in association with unknown molecules, but without concomitant expression of TCR α chain or CD3 chains (30, 31).

In mammals, most peripheral blood lymphocytes (PBL) express αβ TCR, and only 5 to 10% express the γδ TCR (19). However, resident lymphocytes in specific locations, particularly in epithelia, may predominantly express a specific type of TCR (32). For example, in mouse, Thy-1^+ dendritic epidermal cells express predominantly γδ TCR (33-35), and in the gut, the proportion of γδ-bearing T cells is higher than in the blood (36).

The tertiary structure of the TCR heterodimers is not known. This is due to the lack of suitable TCR crystals for X-ray crystallography analysis. This problem may be solved in the near future if the attempts to get enough soluble TCR heterodimers through phosphotidyl inositol - glycan anchors are successful (37). However, a theoretical model of peptide recognition by the TCR has been proposed (3). Using a method analogous to the genetic technique of second-site suppression, contact points between the TCR molecule and the antigenic peptide have been mapped by correlating single aa changes in the peptide with the resulting changes in the TCR molecule. The results of these experiments are consistent with the model predicting a critical role of the CDR3 of the TCR in peptide recognition while more 'external' parts of the TCR molecule would recognize residues on the MHC molecule (21).

The fine stoichiometric structure of the TCR-CD3 complex also remains to be determined. However, recent data suggests that the TCR-CD3 complex contains two functional transducing units formed by the $\gamma\delta\epsilon$ and the ζ molecules (38) and that the CD3 ϵ and CD3 ζ molecules are essential for the expression of TCR complexes, while the remaining CD3 chains may play a secondary role (39).

MOLECULAR GENETICS OF THE TCR GENES

The TCR genes are located in 3 different loci: the α/δ locus, the β locus, and the TCR γ locus. In the mouse, the TCR α/δ locus is located on chromosome 14, the TCR β locus is on chromosome 6, and the γ locus is on chromosome 13. In man, the TCR α/δ locus is on chromosome 14, and the TCR β and γ loci are both on chromosome 7 (40, 41).

The TCR genes have a genomic organization typical of other members of the Ig-gene superfamily that undergo rearrangement, with multiple V genes, D genes for the TCR β and TCR δ loci, and J genes, and one or more C genes (3) (Figure 1).

The TCR α/δ locus is the largest of the TCR loci and extends over a distance of 1000 kb (3, 42, 43). The TCR δ genes are located inside the TCR α locus, in between the Vα and the Jα genes (42-44). The TCR β locus spreads over 700 to 800 kb in most mouse strains (40). However, in some inbred strains of mice, the TCR β locus is shorter as a result of deletion of a considerable number of Vβ genes (45).

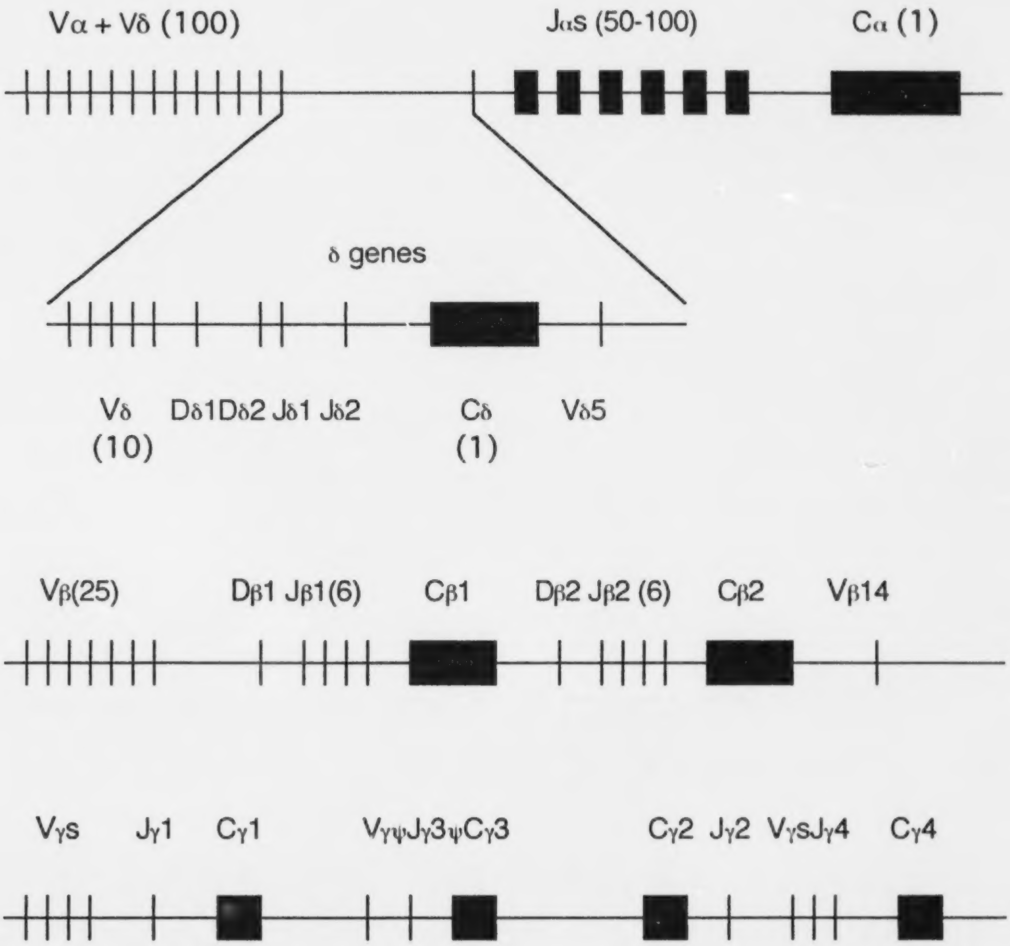
Vα + Vδ (100)
Jαs (50-100)
Cα (1)
δ genes
Vδ
(10)
Dδ1Dδ2 Jδ1 Jδ2
Cδ
(1)
Vδ5
Vβ(25)
Dβ1 Jβ1(6)
Cβ1
Dβ2 Jβ2 (6)
Cβ2
Vβ14
Vγs
Jγ1
Cγ1
VγψJγ3ψCγ3
Cγ2
Jγ2
VγsJγ4
Cγ4

Figure 1.- Genomic organization of the murine TCR genes. The numbers in parentheses correspond to approximate elements for each gene. Genes are represented by vertical bars and black boxes. The graphic is not drawn to scale. ψ: pseudogene.

The V genes are composed of 2 exons: the first exon is a short one that codes for most of the leader sequence. The second exon codes for the rest of the leader sequence and the V region of the TCR polypeptide (40). Some Vβ leader sequences, e.g. Vβ5, are able to undergo variable splicing with a transcript containing a downstream Vβ exon, e.g. Vβ8 (46). Transcripts containing Vβ8 with its own leader segment or with the Vβ5 leader segment are fully functional. This phenomenon has not been reported for other TCR genes or for Ig genes.

For all the TCR loci, the V genes have been grouped in subfamilies under the criterion of nucleotide sequence homology greater than 75% (4). Different numbers of members can be in each subfamily. However, only all the subfamilies of the Vα family have multiple members, while most of the subfamilies for the Vβ, Vγ, and Vδ families have only one member. Estimates of the number of Vα genes range between 50 and 100 genes grouped in 13 subfamilies (4). In contrast, only seven V genes have been reported for the TCR γ and about ten V genes have been reported for the murine TCR δ (19). The TCR β locus contains up to 71 V segments grouped in 21 subfamilies (47-51).

The V genes are usually located 5' to the D or J genes. However, in the mouse, at least one Vβ gene (Vβ14) (52), one Vδ gene (Vδ5) (53 , 54), and two Vγ genes (Vγ1.1 and Vγ1.2) (19) are located at considerable distance 3' to C genes (2.5 to 10 kb depending on the gene). Except for Vγ1.1, the other three genes are in the opposite orientation of transcription and are thought to rearrangeby inversion (see below). Some of the Vδ genes are interspersed with Vα

genes (55) and there is some overlap usage of Vδ with Vα genes (19).

Only the TCR β and δ loci have D genes. For the TCR β genes, only one D gene can functionally rearrange (41). However, for the TCR δ, one or two D genes can rearrange (55, 56). It is interesting to point out that the rearranged D genes for the β or δ loci can be read in all three reading frames, resulting in a significant increase in the diversity of the resulting TCR chain.

The J regions of the TCR are slightly longer than the corresponding regions of the Ig (3). The number of J genes is variable: from two for the TCR δ gene segment to about 50 for the TCR α gene segment.

The C genes of the TCR consist of four exons which represent functionally different domains of the resulting protein. The first exon corresponds to the C region of the TCR chains and codes for an Ig-like domain structure folded into two β-pleated sheets (3, 57). The second exon usually codes for the transmembrane region. The third exon codes for the cytoplasmic domain and part of the 3' untranslated region of the TCR mRNA. Finally the fourth exon codes for the rest of the 3' untranslated region. An alternative exon in the mouse TCR β locus, called Cβ0, has been identified but does not play a significant role in T cell development (58).

For the three different loci, the C genes range from one, as is the case for the TCR α and TCR δ genes, to four, for TCR γ (although one of these is a pseudogene). The location of the C genes also vary. In the TCR α and TCR δ regions, the C gene is located 3' of the last J gene. On the other hand, in the TCR β locus, the C genes are arranged in tandem of one D region gene, six J region genes, and one C gene from 5' to 3'. This organization is conserved in mouse, rat, and human TCR β loci (40, 41, 59). Similar J-C organization has been described for the TCR γ locus. However, this locus has an inverted J-C tandem located 5' to the first Vγ gene (19).

REGULATION OF TCR GENE EXPRESSION

The control of TCR gene expression can be approached at four different levels that reflect four different steps of regulation of gene expression. The first level includes the regulation of TCR gene rearrangement during T cell ontogeny. The second level is the analysis of the cis-acting regulatory elements of each TCR locus and the effect that binding of trans-acting factors to these elements has on TCR gene expression. The third level should approach the mechanism of allelic exclusion by which only one of the two alleles of each active TCR locus is expressed. This phenomenon guarantees the single specificity of the TCR on each particular T cell clone. Finally, TCR expression can be regulated at the post-transcriptional level, in a series of complex interactions involving the expression of other molecules and intracellular events in T cell activation.

CONTROL OF TCR GENE REARRANGEMENT

The organization of TCR loci into V, D, J, and C genes constitutes an essential feature in the generation of diversity of the TCR heterodimer as these genes are able to rearrange in combinatorial joinings (60, 61). In addition to combinatorial recombination, two more mechanisms increase the diversity of the TCR (3). One is based on the fact that the actual joining is imprecise and allows for the joining at three different nucleotides with three different reading frames. The second one is the addition, at the joining points, of nucleotides that are not coded for by the DNA. These additions are known as N-additions and are due to the activity of the enzyme terminal deoxynucleotidyl transferase. In addition to these mechanisms, it has been recently shown that, in the TCR γ and δ gene rearrangement processes, it is possible to have additions of recurrent mono- or dinucleotides, preferentially AT or AG, coded by the DNA of the sister strand (62). These elements are known as P-elements, and they are the result of a particular mechanism of V-(D)-J joining. In contrast to the generation of diversity of Ig, somatic hypermutation has not been described for the TCR.

The rearrangement and expression of the TCR genes starts during T cell ontogeny in the thymus (16, 17, 63, 64). This is a consequence of the expected function of the TCR as well as the processes that immature T cells should go through in the thymus. These include the development of MHC restriction for the TCR, a process known as positive selection, and the deletion of those T cells whose TCR have an unacceptably high affinity for self-MHC, a process known as negative selection which guarantees self-tolerance.

The first TCR genes to start rearrangement and expression are the TCR γ and δ genes (65, 66). This event is first detected around day 14 of fetal life in the mouse, and peaks at day 16. At day 16, TCR β VDJ rearrangement begins to be detectable, and peaks at day 18. This event is followed by rearrangement of the TCR α gene starting at day 16 of fetal life. The rearrangement of TCR genes follows an ordered pattern: first D-J rearrangements occur, followed by V to DJ rearrangements (3). In addition, there are changes in the usage of particular gene elements during life (67-70). This highly specific, sequential expression of the TCR genes may be important in the understanding of the development of MHC restriction and T cell tolerance for the different T cell subsets.

The current model for the rearrangement process involves the joining of the V(D)J genes (reviewed in (61)). This process is primarily governed by the presence of recombination signal sequences (RSS) located at the 3' end of V genes, at the 5' and 3' ends of the D genes, and at the 5' end of the J genes. The V(D)J recombination for TCR genes follows a rule common to the Ig genes and consist of a conserved heptamer separated from a AT-rich nonamer by either 12 or 23 bp. Rearrangement can only occur between RSS that have 12 bp spacers with 23 bp spacer RSS or viceversa, but never with RSS with identical spacer.

The exact nature of the recombination process remains unknown. Based on the biochemistry of the process, it likely involves a recognition activity, an exonuclease activity, an endonuclease activity, a polymerase activity and a ligase activity (61, 71). In addition, it also includes the enzyme terminal deoxynucleotidyl transferase, responsible for the N additions that occur at the junctional points and that adds nucleotides that are not coded by the DNA template.

The recombinase activity is common to B and T cells, as indicated by the fact that TCR constructs can be rearranged in B cells (72), and can be transferred to cells which normally do not express TCR or Ig genes (73). In 1989, Schatz and Baltimore reported the isolation of a gene that activates rearrangement of a recombination substrate (74). This gene, known as recombination activation gene -1 (RAG-1), is expressed in pre B and pre-T cells. The RAG-1 mRNA predicts a highly conserved protein of 119 kd. More recently, another RAG gene, called RAG-2 and linked to the RAG-1 gene, has been isolated on the basis of potentiation of rearrangement of recombination substrates in fibroblasts transfected with RAG-1 (75). RAG-2 does not share aa similarity with RAG-1 or with any other reported protein sequence. The expression of these genes is mostly limited to lymphocyte precursors in thymus and bone marrow. However, using highly sensitive techniques such as the polymerase chain reaction (PCR), it has been shown that brain tissue expresses RAG-1 (76) and that the bursa of Fabricius of chickens express exclusively RAG-2 (77). In addition, the expression of RAG-1 in the brain is probably functional as transgenic mice for a rearrangement substrate fully rearrange this construct in the brain but not in the kidney (78). However, the expression of RAG-1 in the brain may be unrelated to gene recombination or its function may be highly redundant, as mice in which the RAG-1 has been knocked out by homologous recombination do not show any neurological abnormality (79).

The exact function of the RAG proteins is not known. Two hypotheses have been proposed. It may be that RAG genes code for a protein with the enzymatic activities required for the rearrangement process to occur. On the other hand, RAG proteins could be regulatory proteins and control the expression of other proteins involved in the recombination reaction. The present evidence is compatible with both hypotheses.

Recent reports have demonstrated that the expression of RAG-1 and RAG-2 is essential for development of B and T cells. This conclusion is based on the fact that mice in which the RAG-1 or the RAG-2 genes have been knocked out by homologous recombination are severely deficient in B and T cells, with the defect being evident at an early stage of their ontogeny, before rearrangement occurs (79, 80). In addition, RAG-knock out mice are, at present, not leaky for their defect.

The currently accepted model for how TCR and Ig gene rearrangements occur predicts predominantly excision of the DNA between the rearranged gene segments at the base of the stem of the loop formed by the RSS (40). Very recently, double-strand breaks near the RSSs have been demonstrated, providing compelling evidence to support this model (81). However, for those cases in which the gene segment to be rearranged is located in opposite transcriptional orientation, inversion of the gene segment seems the most plausible mechanism (40, 53, 54). It has also been proposed that homologous but unequal sister chromatid exchange may occur in TCR gene rearrangement as partial duplication of the Jβ-Cβ locus has been observed in the TCR β locus of some T cell clones (40). It is not established whether gene conversion can occur during the rearrangement process but this may cause, in part, the allelic polymorphism seen in some TCR loci (82). A similar phenomenon is gene trans-rearrangement which occurs, in vivo, as a result of chromosomal translocation between Vγ and Dδ genes or between Vδ and Jγ genes (83, 84).

Another level of complexity in the understanding of gene rearrangement is the role of chromatin configuration (85). Although, in general, rearrangement of the V(D)J genes for TCR and Ig seems to be essential for full activation of their transcription (86), low levels of transcription from non-rearranged V and J-C regions of Ig (87) and TCR (88, 89) genes occurs prior to the rearrangement process. Similar observations have been made for class switching between Ig heavy chain (H) C gene segment (reviewed in (90, 91). The low level of transcription from non-rearranged genes correlates with future rearrangement of the V and J-C segments (92, 93) or with switching to the particular Ig subclass present in the germline transcripts (94). The expression of these transcripts is independent of enhancer activity (88). However, the upstream region of the transcription starting sites of the Ig H chain C region germline transcripts contains a regulatory element that has enhancer activity in response to cytokines and other stimuli (95).

The significance of the germline transcripts appearing before rearrangement is, at present, unknown. These transcripts may not have any specific function but represent either conformational changes of the chromatin required for the rearrangement to occur or for other totally unrelated events. The finding of DNase I sensitivity of the locus involved in the rearrangement process is consistent with opening of the chromatin but does not tell us anything about their function (72). On the other hand, it is possible that the transcripts themselves are involved in the opening of the chromatin, or participate in directing the recombinase activity to the region (96). Finally, the germline transcripts may code for a protein which is involved in the control of gene rearrangements. This latter hypothesis is supported by the fact that some of the germline transcripts have open reading frames and code for small peptides of 25 to 40 aa (97, 98). However, not all the germline transcripts code for a protein, and it may be that the peptides resulting from these transcript have additional functions (see discussion).

A critical aspect of TCR gene rearrangement and expression is tissue specificity as indicated by restricted expression of functional TCR polypeptides to T cells. Although some transcription from unrearranged J_H-$C\mu$ Ig genes can be detected in T cells (86), no functional Ig chain is produced in T cells. In addition, unrearranged $V\beta$ genes are transcriptionally silent in non-T cells except for some B cell lines.

The mechanisms that determine the appearance of successful rearrangements of TCR genes only in T cell precursors are unknown. They do not involve specific components in gene rearrangement as this process follows similar rules in B and T cells and the recombinase activity is common to B and T cells (72).

It is possible that enhancer-promoter interactions play a critical role in determining tissue-specific expression of TCR genes. This hypothesis is based on the results shown by Ferrier et al (88). Using a murine model of recombination involving a transgenic construct containing an unrearranged TCR VβDβJβ linked to the Ig Cμ gene, these authors showed that partial rearrangements (D-J) occurred in T and B cells only when the construct contained the Ig enhancer but not when this element was not present. The Vβ promoter element was essentially inactive without the enhancer element, as expected from previous reports (99-101). High levels of transcription from the unrearranged Vβ gene of the enhancer-containing construct were seen in the thymus but not in the bone marrow, and complete VDJ rearrangements were almost exclusively seen in T cells. These findings support the concept that an enhancer element is necessary to start the rearrangement process but does not confer tissue specificity on itself. In support of this conclusion, evidence has been presented that the enhancer element of the TCR α locus is methylated in non-lymphoid cells and is undermethylated in both T and B cells (102). Therefore, other elements are involved in providing T cell-specificity to the gene rearrangement event. One of these elements could be related to the V gene promoter. Thus tissue-specific gene rearrangement would be regulated at the level of V to DJ rearrangement by re-locating the right TCR V promoter (with the appropriate DNA conformation, as indicated by the level of transcription obtained from the unrearranged V gene) within the range of action of enhancer elements. Although this hypothesis is based on data from the TCR β genes, it could also apply to the TCR α/δ locus for which the enhancer element is located 69 kb downstream from the most proximal J gene

(42, 43, 101).

A more puzzling phenomenon is the coordination of gene rearrangement and expression of different TCR loci. Introduction of a rearranged TCR β transgene into scid mice turns on expression of non-rearranged TCR α gene, CD4 and CD8 (30, 31). The exact mechanism of this event and whether it involves opening of the chromatin or not is not known, but it implies that the sequential gene rearrangement process observed in T cell ontogeny may be indicative of a regulatory pathway for coordinate expression of TCR loci.

CIS-ACTING REGULATORY ELEMENTS OF TCR LOCI AND THEIR TRANS-ACTING TRANSCRIPTION FACTORS

The second level of control of TCR gene expression is the regulation of TCR gene transcription through the interaction between cis-regulatory elements in the TCR genes and trans-acting transcription factors.

The cis-regulatory elements of the TCR genes can be divided in proximal elements, including promoter regions, and distal elements, which include enhancers and silencers. This organization is similar to that observed in most regulatory regions of eukaryotic genes (103, 104).

Most of our knowledge on the regulatory elements of TCR genes comes from transient CAT assays using constructs with progressive deletions of the region under analysis. Tissue-specificity of enhancer elements is generally tested by using homologous (upstream regions of TCR V genes) or heterologous (regulatory regions from non-TCR genes) promoters or by transfection of the particular constructs into T cells, B cells, or non-haematopoietic cells. More relevant information can be obtained from transgenic animal models in which TCR gene constructs containing different lengths of flanking regions are used.

By analogy with the studies on Ig genes, the initial efforts to map regulatory elements in the TCR genes have been directed to promoter sequences 5' to the V genes and enhancer elements in the introns and 3' of C gene segments of the TCR loci. A general characteristic of the cis-regulatory elements of TCR genes and the trans-acting factors is their high level of organizational complexity with involvement of multiple potential regulatory elements for each gene (105). This feature, however, seems to be the rule not only for TCR genes but for almost any eukaryotic gene (103, 104).

Proximal Regulatory Elements

Careful analysis of the upstream region of different Vα and Vβ genes shows the presence of multiple regulatory elements extending up to 800 bp from the transcription starting site (106-114) (Figure 2). Usually, the TATA box reported has a low degree of homology with the classical consensus sequence (107, 110) and no CAAT box (107, 110) or low homology CAAT sequences (106) have been reported. Functionally, the Vα promoters lack significant transcriptional activity in T cell lines in the absence of the enhancer (100). These features, in addition to the presence of multiple transcription starting sites for V transcripts (110), suggests

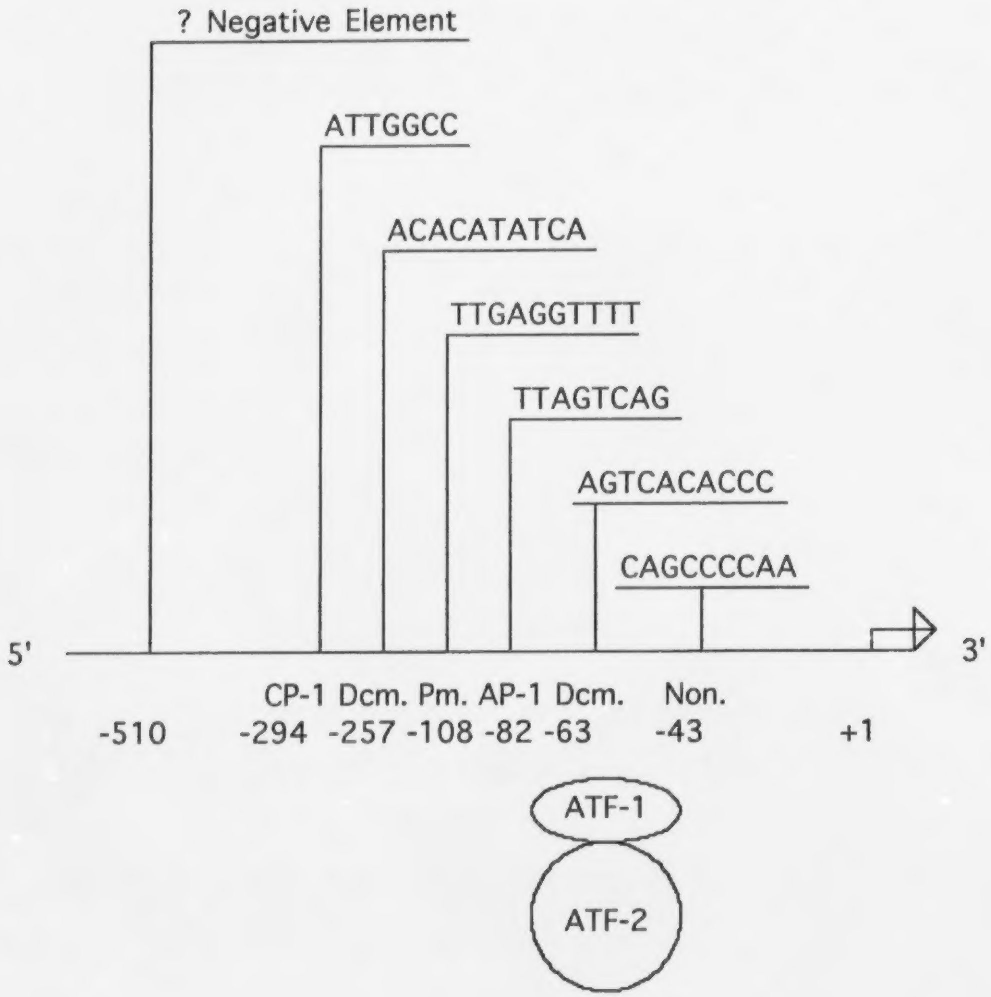
? Negative Element
ATTGGCC
ACACATATCA
TTGAGGTTTT
TTAGTCAG
AGTCACACCC
CAGCCCCAA
5'
3'
CP-1 Dcm. Pm. AP-1 Dcm. Non.
-510 -294 -257 -108 -82 -63 -43 +1
ATF-1
ATF-2

Figure 2.- Upstream proximal cis-regulatory sequences of a murine Vβ gene (not to scale). The two nuclear proteins that bind the decamer motif are also indicated. Dcm.: decamer; Pm.: polyoma; Non.: nonamer.

that the TATA box has poor functional relevance in the regulation of TCR gene expression. This may partially explain the relatively low levels of TCR gene transcription in mature T cells (86).

Common promoters for different members of a Vβ gene family or even members of different families have been reported (110). The implications of this finding may be related to rearrangements of mutually exclusive V genes (109).

In addition to the proximal promoter elements with little similarity to the conventional eukaryotic promoters, other regulatory elements located further upstream of the TATA box have been shown for different Vβ genes (108, 110, 111, 113). Using DNase protection assays of DNA from a human αß T cell line,five footprints representing sites of protein binding to DNA were detected (108). These footprints extended over at least 200 bp upstream of the putative TATA box of the Vβ gene. In addition, four DNase I hypersensitive sites were located in this area. A lymphoid-specific footprint containing a decamer with certain degree of dyad symmetry (AGTGAT/CG/ATCA) and highly conserved in different Vβ families has also been identified (109-111, 113). This decamer motif is located between -63 and -54, binds two nuclear proteins in a sequence-specific manner (with slightly different fine specificities), and is necessary for transcription initiation. Its sequence has some similarities with the cAMP-responsive element (CRE) and the AP-1 binding sites (110, 112). Although binding to the decamer has been reported for specific thymic factors (70), some other proteins that bind this decamer motif are present in lymphoid and non-lymphoid nuclear extracts. Two nuclear proteins that bind the decamer, called TCR-ATF1 and TCR-ATF2, belong to the bZip class of proteins, containing a leucine zipper motif and a basic region, and have high degree of homology with other proteins known to bind CRE such as CREB, hXBP1, mXBP1 c-JUN, and c-Fos (115). In addition, the decamer motif confers cAMP inducibility of transcription (115).

An AP-1 binding site has been detected in between -82 and -75, implying some inducibility of TCR β gene transcription after signal transduction and phorbol-ester-induced stimulation (113).

Studies using transfection of constructs containing different regulatory regions located further upstream to the transcription starting site (up to -343) showed the importance of additional elements. When the constructs contained only the region between -85 to +1 of the Vβ promoter linked to the CAT gene, CAT activity was detected only in T cells and fibroblasts but not on B cells. However, a T cell-specific enhancement of transcription of the CAT gene was seen when the construct contained the elements contained in between -85 and -343 (111, 113). This finding points out that elements that positively control full T cell-specific TCR β gene transcription lie upstream of the proximal promoter region of the TCR Vβ genes. These elements have been called T cell promoter elements (TPE) and are transcriptionally silent in B cells, either because of a negative trans-acting factor or a lack of positive trans-acting factor. The -82 to -343 region corresponds partially to the other footprints detected by Royer and Reinherz (108) and contains a consensus sequence for a polyoma-like enhancer (from -107 to -97), an inverted repeat (from -170 to -151), a direct repeat (from -257 to -248) and a CAAT box-like element (from -294 to -288) (113).

An additional element with negative effects on TCR Vβ transcription has been mapped to a 60 bp sequence located in between -510 and -450 (111, 113). This element is active on homologous and heterologous promoters but only on T cells (111). When the TCR β enhancer element is present, the activity of this negative element disappears (113).

Enhancer Elements

The search for enhancers in TCR loci was originally concentrated to the J-C introns of TCR loci. In particular, enhancer elements were initially reported in the Jα-Cα intronic segment of the human TCR α/δ locus (107), the Jβ2-Cβ2 intron of the murine TCR β locus (116), and the intron between Jδ3 and Cδ of the human TCR α/δ locus (117). The intronic TCR α enhancer was further claimed on the basis of the presence, in the Jα-Cα region, of a duplicated decamer separated by 35 bp with high degree of homology with the IgH chain enhancer (107). For the TCR β locus, DNase I hypersensitive sites are consistently detected in the Jβ2-Cβ2 intron (118, 119), and binding sites for NF-κB and NF-βi2 are present in this region (120). However, the intronic enhancers for the TCR α and β loci have not been confirmed in this location by other groups neither in human or mouse (99-101). The reasons for these conflicting results are not clear.

For the murine and human TCR α/δ locus, a set of potent enhancer elements have been mapped 3 to 4.5 kb downstream to the Cα gene segment (100, 101). These enhancer elements share 90% sequence identity (105). For the murine TCR α locus, the enhancer activity is located in a region of 230 bp which has four sites (NF-α1, NF-α2, NF-α3, and NF-α4) able to interact with DNA-binding proteins (101). The NF-α2 and NF-α4 binding sites are essential for enhancer activity, while NF-α3 and NF-α4, which share a general sequence of a pyrimidine stretch followed by a purine stretch, are apparently T cell-specific.

The human TCR α enhancer elements have been mapped to a 275 bp region 3' to the Cα gene segment (100) (Figure 3). Footprinting analysis of this region showed four functionally significant binding sites for DNA-binding proteins, called Tα1, Tα2, Tα3, and Tα4; a fifth site, Tα5, did not add any significant enhancer activity (100). Tα1 corresponds to the mouse NF-α2 site. The Tα2 site, which was originally described as containing two protein-binding sites and is now known to have three different binding sites, corresponds to the murine NF-α3 and NF-α4 sites. The equivalent murine sites to the human Tα3 and Tα4 sites have not been identified because the construct used by Winoto and Baltimore (101) did not extend beyond 3' of the murine equivalent to Tα2 (105).

The human TCR α enhancer element is preferentially active in αβ T cells, and is also active on heterologous promoters. The Tα1 and Tα2 sites, that extend a region of 116 bp, are sufficient for minimal enhancer activity, while the Tα3 and Tα4 sites alone have ten times less activity than the Tα1 and Tα2 site-containing construct (100). However, the presence of Tα3 and Tα4 sites may compensate for

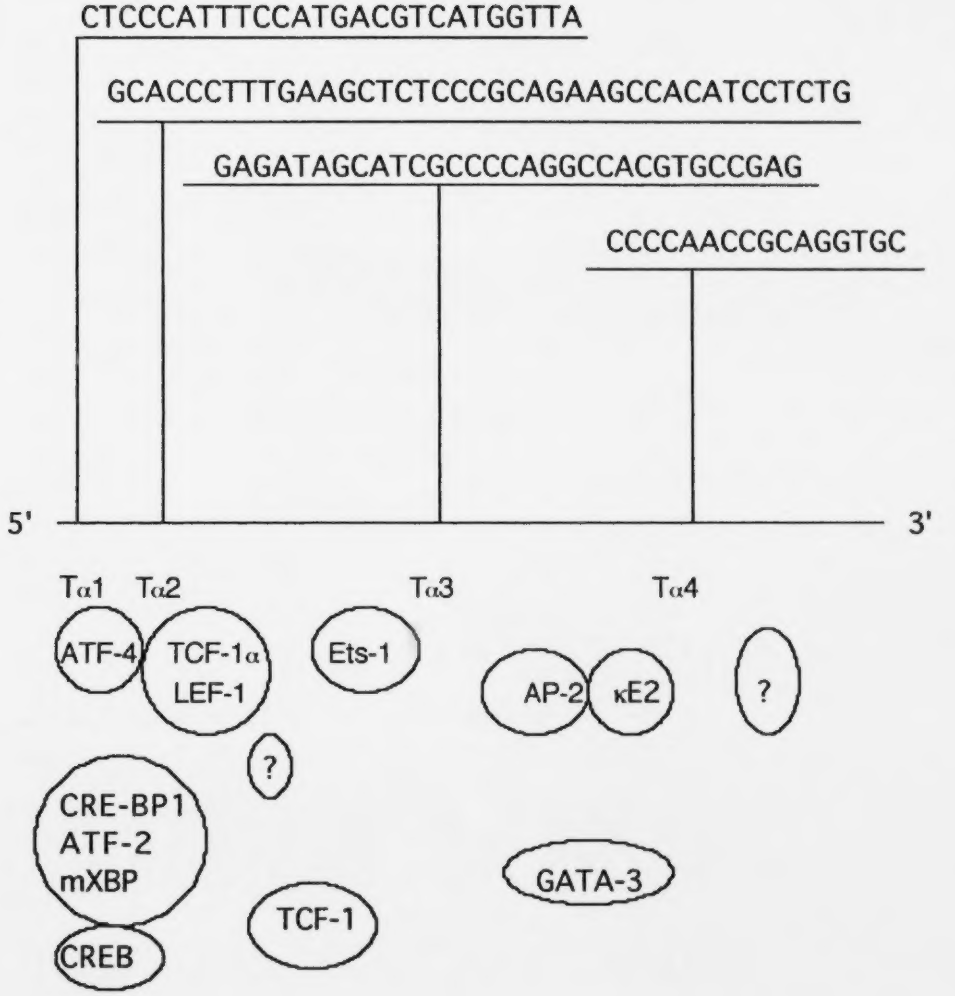
CTCCCATTTCCATGACGTCATGGTTA
GCACCCTTTGAAGCTCTCCCGCAGAAGCCACATCCTCTG
GAGATAGCATCGCCCCAGGCCACGTGCCGAG
CCCCAACCGCAGGTGC
5'
3'
Tα1
Tα2
Tα3
Tα4
ATF-4
TCF-1α
LEF-1
Ets-1
AP-2
κE2
?
?
CRE-BP1
ATF-2
mXBP
GATA-3
TCF-1
CREB

Figure 3.- Organization of the human TCR α enhancer and the putative transcription factors binding to the different enhancer elements. Modified from (105).

mutations that abolish the function of the Tα1 and Tα2 sites (102). The 20 bp spacing between Tα1 and Tα2 sites, but not their nucleosome phasing, seems to be critical for enhancer activity.

The sequence of the Tα1 site shares homology with the CRE and binds four to six CRE binding (CREB) proteins, three of which have been characterized as CREB, CRE-BP1 (also known as ATF-2 or mXBP), and ATF-4. None of these proteins are T cell-specific (105). CREB protein confers cAMP-responsiveness, while CRE-BP1 confers responsiveness to the viral E1A protein. The function of ATF-4 is unknown but it may act as a repressor of CRE-dependent transcription in certain cell types.

The Tα2 binding site does not have any sequence similarity with previously reported protein-binding motifs. The Tα2 site binds at least four different proteins, three of which have been identified and characterized: murine and human TCF-1 (121, 122), TCF-1α or LEF-1 (123), and Ets-1 (124). TCF-1 is a lymphoid -specific, and perhaps T cell-specific DNA binding protein with a high mobility group (HMG) domain, and also binds to the CD3-ε enhancer element (121). The HMG domain is essential for DNA binding. However, in contrast to other proteins with an HMG domain, such as HMG-1, TCRF-1 binds DNA in a sequence-specific manner. LEF-1 is also a DNA binding protein with an HMG domain which is expressed in αß and γδ T cells, during all stages of development. However, the activity of TCF-1 and LEF-1 seems to require the presence of other transcription factors. Ets-1 binds to the 3' end of Tα2 (124) and is preferentially expressed in thymocytes and T cells

(125). In thymocytes, the time course of expression is almost identical to the time course of expression of TCR α gene. However, Ets-1 alone is not able to trigger TCR α gene expression. In addition to Ets-1, another Ets-related protein has been recently identified. This protein, called Ets-4, is able to bind both TCR α and TCR β enhancer elements (105). It is interesting to note that the Tα2 site may act as an activator or as a repressor of transcription depending on the DNA regulatory regions surrounding it (126).

The Tα3 site binds at least three different proteins. Close examination of Tα3 reveals a GATA box, an AP-2-like site, and a κE2 (E box)-like site (105). The GATA box binds a protein known as GATA-3 (127, 128) which is a member of the family of GATA proteins. These proteins play an important role in haematopoietic cell development (129-131). The chicken, murine and human GATA-3 are highly homologous, and have two Zn finger domains (127, 132, 133). The pattern of expression of GATA-3 is particularly interesting as it is expressed predominantly in αß and γδ T cells but not in other haematopoietic cell lineages (134). The time course of GATA-3 expression during T cell ontogeny is consistent with a role of GATA-3 in determining cell maturation to T cell lineage, as it is expressed very early in thymocyte maturation (105). In addition, GATA-3 expression can be up-regulated with PMA, and this event is associated with expression of TCR α mRNA in an immature T cell line which does not normally express this gene (127). However, GATA-3 expression is found in the brain and in the kidney of chickens and in murine brain and placenta (132, 133). In chicken kidney, there are two forms of GATA-3 mRNA (132).

Finally, the Tα4 binding site does not have any sequence similarity with previously reported protein-binding motifs, and binds three ubiquitously expressed nuclear proteins, still uncharacterized (105).

The mouse TCR β enhancer has been mapped 3' to the Cβ2 gene segment (99, 135), in a region containing two DNase I hypersensitive sites located 4.5 and 3.3 kb downstream of Cβ2 (118). Both sites are present only in αβ T cells but not in B cells or fibroblasts. Only the distal DNase I hypersensitive site is present in T cell precursors, suggesting that the proximal site to Cβ2 is not only T cell-specific but also stage-specific. Both sites are necessary for TCR β gene transcription as shown in transgenic mice (135). The presence of this hypersensitive region corresponds to the location of important regulatory elements as shown by sequence homology of these sites to the human TCR α enhancer, the SV40 enhancer, the polyoma virus enhancer, the IgH enhancer sites, and AP-1-like binding sites (99, 118, 135). More recently, careful DNase I footprinting analysis and gel mobility shift assays have shown that, in an area of 695 bp in this hypersensitive region, there are seven protein-binding sites, known as β1 to β7 (136). The βE2 site is similar to the Vβ decamer motif. The βE3 site has homology with the κE2 sequence and the βE4 site has homology with the SV40 enhancer. On the other hand, the βE6 is identical to the μEBP-E motif of the Ig enhancers, and also contains sequence homology with the polyoma virus enhancer. Binding to these sites could be competed with the presence of the decamer motif present in the Vβ promoter region or the Ig enhancer κE2. The murine TCR β enhancer is inducible by PHA and PMA as suggested by the presence of AP-1-like binding sites (136). It is interesting to point out that T cell-specificity of this minimal enhancer element is only achieved by including 4 kb of flanking regions, suggesting that the in vivo requirements may be more complex than the in vitro requirements. This possibility is also valid to other systems such as the regulation of MHC class II gene expression (137).

The human TCR β enhancer element has been analyzed recently (138). This enhancer element is similar to the murine counterpart, and contains sequences with homology to the Ig κE2 motif, CRE, and AP-1 binding sites. The enhancer is predominantly active on αβ and γδ T cells, but is not active in fibroblasts. The minimal enhancer activity is located in a 480 bp region mapped 3' to Cβ2, containing five protein binding sites (Tβ1 to Tβ5), of which Tβ2, Tβ3, and Tβ4 are essential.

Analysis of the murine and human TCR β enhancer indicated that the TCR α and the TCR β enhancers share binding proteins, such as a CRE-binding protein in Tα1 and Tβ2, binding of TCF-1 to Tα2 and Tβ5, and binding of Ets to Tα2 and to Tβ3 (105, 138). In addition, a GATA box has been reported in Tβ2 (105) and binding of GATA3 to this box has been demonstrated (128). These findings suggest that the regulation of the expression of TCR α and TCR β genes is coordinated. However, there is also evidence that expression of TCR α and TCR β genes may be under different control mechanisms. The first evidence for such differential control was provided by MacLeod et al (139). Using somatic cell hybrids between a T cell lymphoma line with no TCR β gene expression and another T cell lymphoma line expressing a functionally rearranged TCR β gene, these authors showed that the resulting hybrid did not express TCR β mRNA, while the expression of TCR α mRNA was not affected. The lack of expression of TCR β gene was not due to chromosomal loss, and could be reverted with cycloheximide. From these results, they concluded that an specific negative trans-acting factor was involved in turning off the expression of the TCR β gene. These results imply, first that gene rearrangement, although necessary for full transcription is not sufficient, and second, that TCR α gene expression does not require TCR β gene expression, arguing against previous suggestions made on the basis of ordered rearrangement of TCR genes (65, 66). More recently, using a T-lymphoma cell line which does not express functional TCR α or TCR β mRNAs, it has been shown that, although protein synthesis inhibitors induce a reversible increase in the amount of functional TCR α and β mRNAs, these agents induce transcription of only the TCR β gene but not of the TCR α gene (140).

The differential control of expression of TCR α and TCR β genes applies also to different elements responding to different pathways of signal transduction. For example, calcium ionophores increase TCR α gene transcription in the absence of any increase of TCR β transcripts (140), while phorbol esters induce an increase in transcription of both TCR α and TCR β genes (141, 142). However, phorbol ester-induced TCR α gene transcription is independent of new protein synthesis while the increase in TCR β gene transcription requires new protein synthesis. The differential control of TCR gene expression by different signal transduction pathways also applies to the TCR γ genes (142). Together, this evidence further corroborates that, in certain circumstances, there is differential regulation of TCR α and TCR β gene transcription. The reason for such finding is not clear yet but it may have to do with different mechanisms of control during the ordered sequence of rearrangement and expression of TCR genes during T cell ontogeny, or, alternatively, that one of these genes has additional functions requiring its separate expression.

Much less is known about the regulatory sequences and trans-acting DNA-binding proteins for the TCR γ and TCR δ genes. In the murine TCR γ locus, an enhancer element has been located in a 3.9 kb region located 2.5 kb downstream of Cγ1 (143). This region contains six hypersensitive sites (NF-γ1 to NF-γ6), of which three (NF-γ2, NF-γ3, and NF-γ4) are essential for enhancer activity. The minimal enhancer activity can be located in a 200 bp region containing these three sites. The TCR γ enhancer works in a T cell-specific manner, although it does not work in all T cell lines if it is not present in multimerized form (143). This suggests that this enhancer has a relatively weak activity and requires the presence of other elements to achieve full activity. In addition, this minimal enhancer element contains several motifs with homology to enhancer elements in other TCR genes and Ig genes, and viral enhancer elements. It is interesting to point out that NF-γ1, NF-γ3, and NF-γ4 have homology with the Tα2 element, the

TCR α NFα5 element, the TCR β core enhancer, and the TCR δ E3 element (see below). The NF-γ1 site has homology with the GATA3 binding site but binding of GATA3 to this site could not be demonstrated (128). In addition, the TCR γ enhancer element contains regions of homology with AP-2 binding elements, topoisomerase II binding sites, and matrix associated regions.

An interesting feature of the regulation of expression of the murine TCR γ gene is the fact that, in pre-B and pre-T IL-3-dependent cell lines, the presence of IL-3 in the medium induces transcription of non-rearranged TCR γ gene (144). The withdrawal of this cytokine greatly reduces the presence of TCR γ transcripts and this is not due to cell death. The implication of this result is that cytokine-induced transcription from unrearranged TCR genes likely occurs during lymphocyte development, as IL-3 acts on early stages of differentiation (145), and that cytokines may have an important effect in regulation TCR gene transcription (16). However, to my knowledge, no other report has addressed the specific effects of cytokines in TCR gene transcription. Nevertheless, there is substantial evidence that cytokines regulate Ig gene transcription and class switching (95, 146, 147).

At the present time, nothing is known about the organization and function of the human TCR γ enhancer.

The murine and human TCR δ enhancers are, so far, the only TCR enhancer elements located in a J-C intron, specifically in the Jδ3-Cδ intron in humans (117, 148), and the Jδ2-Cδ intron in the mouse (149). The human TCR δ enhancer is mapped in a 1.4 kb region with maximal enhancer activity located in a 250 bp segment near the 3' end of this region. The 250 bp segment contains seven protein-binding sites (δE1 to δE7), of which two (δE3 and δE4) have very potent enhancer activity and are the core of the enhancer element, and one of them is essential (δE3) (150). The δE3 and part of the δE4 sites share homology with the κE2 site while the δE1 and δE4 sites have GATA boxes. In addition, binding of GATA3 to the δE4 has been demonstrated in vitro (128, 133, 134). The δE2, δE3, δE5, and δE7 sites have homology with Tα2 of the TCR α enhancer, and, in particular, the δE7 is identical to the Tα2 TCF-1-binding site (148). More recently (150), a new δE3-binding protein, called NF-δE3A has been isolated. This protein is predominantly expressed in $\gamma\delta$ T cells and in $\alpha\beta$ T cells in which exhibits some low affinity binding with Tα2. However, it only has low affinity binding with the μE3 regulatory sequence of Ig genes. It is interesting to point out that a 400 bp 5' flanking region of the minimal TCR δ enhancer element may contain a weak negative regulatory element, and that δE1 also has a weak negative regulatory activity on the TCR δ enhancer (117, 148, 150). The human TCR δ enhancer element is active on $\alpha\beta$ and $\gamma\delta$ T cells and promotes transcription from homologous and heterologous promoters.

The mouse TCR δ enhancer is not as well characterized as the human counterpart (149). It is located in the Jδ2-Cδ intron, in a 766 bp region with high degree of homology with the human TCR δ enhancer, implying that it may have similar functional properties.

From the data presented so far, it is evident that there is a building pattern for enhancer elements of TCR genes as well as other T cell specific genes such as the CD3δ and CD3ϵ genes, the interleukin 2 gene, and the CD2 gene (17, 105). This is consistent with the finding that multiple transcription factors in T cells can coordinately regulate T cell function at the level of T cell-specific gene transcription. Such level of redundancy, with still some possibility of differential expression of the genes involved could be explained by three different explanations (105). It is possible that the function of the different transcription factors in specific sets may lead toward different patterns of gene expression. This possibility is consistent with the finding that some transcription factors require the presence of other trans-acting factors in order to be active. On the other hand, it is possible that some transcription factors display different binding specificities under different circumstances and by different unknown mechanisms. This alternative is particularly attractive when considering the Ets family of transcription factors, in which binding capacity of some of its members to particular DNA sequences seems to depend on the conditions of the experiment. Finally, some regulatory elements may have negative effects on the expression of particular genes, or even have dual effects depending on the location in the genome. This is consistent with the description of silencers in the TCR α/δ and γ loci (see below), as well as the dual properties of the Tα2 binding site in the TCR α enhancer (126).

In addition to positive control elements in the TCR gene regulatory region, negative cis-acting elements, known as silencers, have been recently reported (151, 152). These elements have been found in the TCR α/δ locus and in the TCR

γ locus. The main function of these elements is to ensure transcriptional inactivity in cells of different lineage (153). The TCR α silencer is located 5' to the TCR α enhancer and consists of at least two elements with sequence homology with topoisomerase II sites and matrix-associated regions (151). The α silencer is responsible for turning off transcription of the TCR α gene in non-$\alpha\beta$ T cells, and is also active in non-T cell lines. Another silencer element has been described for the TCR γ locus (152). Its function is similar to that of the TCR α silencer, as it shuts off the expression of the TCR γ gene in non-$\gamma\delta$ T cells. In the absence of this silencer, transgenic mice for the $\gamma\delta$ TCR have a severe blockade of $\alpha\beta$ T cell maturation (153). In its presence, $\alpha\beta$ T cells develop normally.

CONTROL OF TCR MONO-SPECIFICITY IN EACH T CELL: ALLELIC EXCLUSION

Peripheral T cells express a single type of TCR that, except for some rare exceptions (29), is either an $\alpha\beta$ or a $\gamma\delta$ TCR (2). Moreover, all the TCR molecules expressed on the surface of a particular T cell have the same Ag specificity. This feature is consistent with the clonal selection theory that predicts clonal expansion of those T cells with a particular Ag specificity, after engagement with MHC-antigenic peptide complexes (18). The mechanisms that control the expression of a particular TCR heterodimer in any given T cell are not entirely clear. They may involve the preferential expression of certain TCR loci, the preferential association of particular TCR molecules, and a mechanism of allelic exclusion.

The preferential expression of particular TCR loci in a given T cell is a question closely link to the lineage relationship between $\alpha\beta$ and $\gamma\delta$ T cells. Whether TCR gene rearrangement follows a sequential, stochastic model or $\alpha\beta$ vs $\gamma\delta$ TCR gene rearrangement is the result of an ordered, pre-determined state is a matter of debate (64). Analysis of the DNA circles resulting from excision of intervening DNA in the TCR α/δ locus of $\alpha\beta$ T cells has yielded controversial results, as in some cases the TCR δ gene in the circles was still in germline configuration (154) and, in other cases, was rearranged (155), indicating that $\alpha\beta$ T cells developed from unsuccessfully rearranged $\gamma\delta$ T cell precursors. A more informative strategy has been the use of transgenic mice for TCR $\gamma\delta$ (153). When the transgenic constructs did contain a TCR γ silencer, $\alpha\beta$ T cells developed normally. In the absence of the TCR γ silencer, there was a severe blockade of $\alpha\beta$ T cell maturation. This result strongly supports the idea that $\gamma\delta$ and $\alpha\beta$ T cells are the result of two different cell lineages. Silencer elements have been also detected for the TCR α genes and, in this case, the silencer may repress the activity of the TCR α enhancer in non-$\alpha\beta$ T cells (151). However, it has been shown that $\alpha\beta$ T cells may have rearranged TCR γ loci, although in these cases, there is no transcription from the rearranged TCR γ locus (65). More recently, the analysis of the enhancer elements of the different TCR loci has shown that some cis-regulatory regions and some transcription factors are shared by members of different TCR loci. This finding has been taken as evidence in favour of a sequential model of T cell lineage determination in which the differentiation into $\alpha\beta$ vs $\gamma\delta$ T cells would be decided at the level of gene transcription (148).

The preferential association among different TCR chains has been elegantly shown by Saito et al (22). Using transfected cells expressing functional TCR α, β, γ, and δ proteins, they showed that TCR α and β chains and TCR γ and δ chains associated in a mutually exclusive manner. No mixed heterodimers, such as $\beta\delta$ or $\alpha\gamma$, were detected. The mechanisms involved in such preferential association are not known, but likely also involve the interactions of each TCR chain with specific CD3 chains in cooperative or inhibitory sense (156).

As for the Ig genes, it seems that allelic exclusion is operating in TCR gene expression but it is not known yet whether allelic exclusion follows an stochastic model, by which any one of the chromosomes for a particular TCR gene could start rearrangement, or a regulated model in which functional rearrangement occurring at one locus would suppress rearrangement at the other locus. Nevertheless, the evidence for allelic exclusion comes from two different types of experiments. First, sequencing of TCR loci in both chromosomes has shown that, in most cases, only one of the loci is functionally rearranged while the other is either in germline configuration or in an out-of-frame rearrangement (109). This is particularly true for TCR β genes. The data regarding the TCR α locus is not as clear, as secondary and tertiary functional rearrangements of the TCR α locus have been described (89, 157). The second line of evidence for allelic exclusion in TCR gene expression comes from the analysis of transfected cell lines or transgenic mice for TCR genes (30, 158-163). These experiments have shown that introduction of a functionally rearranged TCR gene usually suppresses the rearrangement and expression of endogenous TCR genes, with evidence that this effect is mediated through the protein resulting from the transgene (160, 161). From these experiments, however, some differences in the mechanisms involved in allelic exclusion of TCR α and TCR β genes became evident. While transgenic mice for the TCR β chain show no rearrangements for endogenous TCR β gene, whether or not the construct contains an enhancer element, transgenic mice for an $\alpha\beta$ TCR show rearrangement for the endogenous α when the TCR α transgene does not contain an enhancer element (158, 164, 165). However, endogenous TCR α gene rearrangements are almost completely abolished when the TCR α transgenic construct contains an IgH enhancer element (160, 164, 166). In contrast, recent evidence indicate that in cloned thymocytes of transgenic $\alpha\beta$ TCR mice, rearrangement of endogenous TCR α genes only stops after positive selection in the thymus and this occurs in spite of the presence of a TCR α transgene (167). This would imply that it is possible for some T cells to express two different TCR α chains, and because this is unusual in the periphery, it leads to suggest that allelic exclusion for TCR α could occur at the level of the protein.

In summary, allelic exclusion in T cells probably represents a common phylogenetic mechanism to ensure single specificity for the receptor expressed on a particular cell. However, analysis of those cases in which allelic exclusion is not followed, may reveal new interactions between TCR proteins and between TCR proteins and cis-regulatory elements in the TCR loci.

POST-TRANSCRIPTIONAL REGULATION OF TCR GENE EXPRESSION

Post-transcriptional regulation of gene expression is still largely unknown. This also applies to the regulation of TCR gene expression. On the basis of lack of data on post-transcriptional regulation of TCR gene expression, it is widely assumed that most regulatory events for TCR expression occur at the level of gene transcription. However, some isolated reports indicate that post-transcriptional regulation of TCR gene expression may be an important step regulating TCR expression on the cell surface.

In 1988, Wilkinson and MacLeod reported that cycloheximide induces an increase in steady-state RNA levels for TCR α and TCR β (140). These authors concluded that the cycloheximide-induced increase in TCR α mRNA was due to a reversal of nuclear post-transcriptional events and/or cytoplasmic events related to RNA stability, as cycloheximide did not increase TCR α gene transcription. In contrast, cycloheximide induced an increase in TCR β gene transcription as well as an increase in steady-state RNA levels. The mechanism responsible for this phenomenon is not known. It may be that cycloheximide blocks the expression of a protein that represses TCR β gene expression, or that cycloheximide decreases the levels of a protein involved in RNA degradation.

More recently, post-transcriptional regulation of TCR expression has been suggested by the demonstration of inverse correlation between steady-state levels of TCR mRNAs and levels of surface expression of TCR (256). In addition, it has been shown that TCR expression is downregulated very early (between 4 and 7 hours) after T cell activation with mAb against CD3 plus PMA or with mAb against CD2 (168). The downregulation of TCR expression is due to a decrease in TCR α and TCR β gene transcription as well as a decrease in the half-life of RNAs for both TCR chains. The initial down regulation of TCR α and β expression is followed by an induction of their expression with an increase of RNA stability occurring first and being followed by an increase in transcription. The kinetics of down-regulation followed by upregulation of TCR α and β genes were also observed for TCR γ, CD4, and CD8. Nothing is known about the molecules involved in affecting RNA stability in this situation. However, downregulation of TCR and CD8 expression could play a crucial role in the induction of extrathymic tolerance (169).

Post-translational regulation of TCR expression has been shown in double positive thymocytes after in vivo treatment with anti-CD4 mAbs. The effect of administration of mAb against CD4 on T cell development is the practical elimination of $CD4^+$ single positive cells in the thymus (170). In the double positive thymocytes of anti-CD4-treated mice, there is an increased expression of TCR on the cell membrane. The increase in expression is due to an increase of release of newly synthesized TCR chains from the endoplasmic reticulum, and not due to increases in transcription, in translation, or in assembly (171).

Finally, the expression of TCR molecules may be regulated by availability of CD3 molecules. In particular, transfection experiments have shown that the CD3

ζ and the CD3 ϵ chains are critical for the expression of functional TCR complexes on the cell surface (172).

OBJECTIVE OF THIS THESIS

During studies on the T cell repertoire in a murine model of autoimmune glomerulonephritis (173), we detected, in kidneys of normal mice, an RNA that hybridized with a TCR α cDNA probe. The size of the detected RNA was sightly smaller than the size of a complete TCR α mRNA (1.3-1.4 kb vs 1.6-1.7 kb). The purpose of this thesis is the characterization of the expression of such an unusual transcript at the molecular and cellular levels.

The characterization of the cellular origin of the truncated TCR α mRNA should be approached under the consideration of the particular features of the kidney cell populations, that is, epithelial vs interstitial cells. Due to the particular pattern of TCR gene expression, the experimental work related to the characterization of the cells expressing the truncated transcript should include the testing of their origin, the relationship between these cells and the T lymphoid lineage, their thymus dependence, and their need for intact recombination capability.

The molecular characterization of the truncated TCR α mRNA involves the analysis of the transcript itself as well as the molecular biology of the transcript which includes processing and translation.

The potential relevance of pursuing such finding is two-fold. On one hand, if the transcript is, indeed, a truncated, germline TCR α mRNA, then the expression of such transcript in the kidney may be a useful model to study in vivo control of TCR α gene rearrangement and expression, and the role of germline transcripts in the recombination process. As the control of the expression of TCR α gene is a good example of developmentally regulated, tissue-specific gene expression, some insights in the molecular events determining this process may be gained.

Another potentially relevant aspect of this project is that the expression of the truncated transcript may be a good indicator to define a novel subset of renal interstitial cells. Little is known about the resident cell populations in the renal interstitium and their functions. A better understanding of their cell lineage as well as their phenotypic identity may be useful to pursue other research questions that are currently hampered by the lack of clearly defined experimental models.

CHAPTER TWO:

MATERIAL AND METHODS

MICE

Eight to twelve week old male and female CBA/J mice (H-2^k), DBA/2J (H-2^d), BALB/cCrAlt (H-2^d), BALB/c nu/nu and their normal littermates nu/+ (H-2^d), C3H/HeSnJ and C3H/HeSnJ scid/scid (H-2^k) were purchased from the Jackson Laboratory (Bar Harbor, ME). In addition, mice congenic for the Ly-6 gene (A.Ly-6.2; H-$2^{k/d}$) were bred in the animal facilities of the University of Alberta. Mice were maintained in our animal colony with standard mouse chow and free access to water. Mice were killed by neck dislocation, and organs were harvested after exsanguination and snap frozen and stored at -70^{0}C or placed in phosphate buffered saline (PBS) for immediate use. Tissues for histological studies were embedded in Tissue-Tek O.C.T. (Ames Division, Miles Laboratories Inc, Elkhart, In), snap frozen, and stored at -70^{0}C.

RENAL CELL FRACTIONATION

Renal tissue fractions enriched in interstitial cell populations were obtained using the technique reported by Schreiner et al (174), with some modifications. Briefly, 10 to 20 kidneys were minced and sieved through a 150 μm sieve in Hank's balanced salt solution (HBSS) (Gibco Laboratories, Grand Island, NY). The cells were washed twice in HBSS (2500 r.p.m. for 20 min. at room temperature) and the pellets underwent enzymatic digestion with HBSS containing 1% Hepes buffer, type II collagenase (Sigma Chemical Co., St. Louis, MI) (500 μg/ml) and DNase I (Sigma Chemical Co., St. Louis, MI) (0.01 mg/ml) for 30 minutes. After digestion, cells were spun down and washed twice (2500 r.p.m. for 10 min. at 4^{0}C) with PBS with 2% fetal calf serum and placed in PBS with 1 mM EDTA for 10 minutes. After centrifugation and one wash in PBS, the suspension was allowed to settle for 1 minute, the supernatant was collected, and the cells were washed and resuspended in PBS. At this time, the cell suspension contained interstitial cells, red blood cells, and some intact glomeruli and small tubule epithelial cell clumps. Further separation was performed with Percoll gradients from <40% to 100%, and cells were pooled in four fractions: <40%, 40-50%, 50-70%, and 70-100%. The <40% fraction contained large cells with some glomeruli and tubule epithelial cell clumps. The 40-50% contained macrophage-like cells and some lymphocytes, while cells in the 50-70% fraction were mostly lymphocyte-like. The 70-100% fraction was mainly composed of red blood cells. The percentage of cells isolated in each fraction in relation to the total number of cells isolated from each kidney is represented in Figure 4.

RNA EXTRACTION AND NORTHERN BLOTTING

Total RNA from frozen kidneys was extracted using the guanidinium - cesium chloride method (175). Total RNA from each cellular fraction was extracted by the technique reported by Chomczynski and Sacchi (176). Cytoplasmic RNA from solid organs was extracted following the technique reported by Bothwell et al (177), with some modifications (175). Briefly, cell lysis (from 2 kidneys) was performed in 2 ml of lysis buffer (50mM Tris-HCl pH 8.0, 140 mM NaCl, 1.5mM

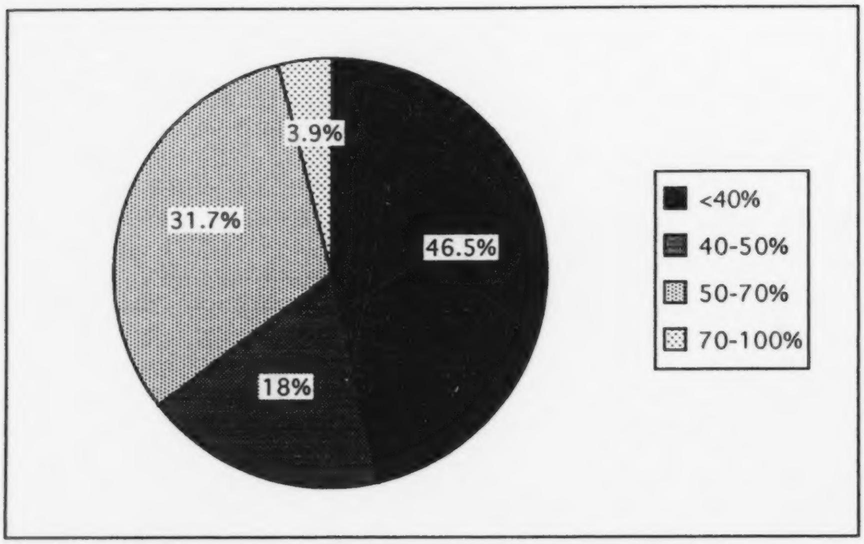

Figure 4.- Cells isolated in each fraction as percentage of total number of cells isolated from each kidney. The results are presented as mean of 8 cell separation experiments. The standard deviations for these experiments were: 14.2% for fraction <40%, 6.3% for fraction 40-50%, 12.7% for fraction 50-70%, and 2.4% for fraction 70-100%.

$MgCl_2$, 0.5% Nonidet P-40) in the presence of 20 mM Vanadyl Ribonucleoside Complex (Gibco BRL, Gaithersburg, MD). After pelleting the nuclei (2000 r.p.m. for 5 min at 4^0C), the supernatant was collected and added to 4 ml of 6M guanidinium thiocyanate and 32 μl of β-mercaptoethanol. The samples were then spun down (10000 r.p.m. for 10 min at 4^0C) and the supernantant processed as for extraction of total RNA following the cesium chloride method (175). Poly A^+ RNA was isolated by oligo-dT cellulose column chromatography (Boehringer Mannheim GmBH Canada Ltd., Laval, Quebec) with a poly-A^+ recovery of 1-2% of the total RNA applied (175).

The final concentration of RNA was measured by spectrophotometry at absorbance of 260 nm. Northern blot analysis was performed using 5.5–30 μg of RNA for each sample, run in a 1.5% agarose, 2.2 M formaldehyde gel and transferred to a nitrocellulose filter. Filters were incubated in pre-hybridization solution containing 50% formamide, 5X Denhardt's solution, 5x SSPE, 0.1% SDS, 100 μg/ml of denatured salmon sperm DNA, and 5% dextran sulfate, for 4 to 6 hours, at 42^0C (175). The filters were then hybridized with random oligo-primed ^{32}P–labelled cDNA probes for murine TCR $C\alpha$ (178), murine TCR $C\beta$s (179), murine TCR δ (180), I-$A\alpha^d$ (181), and murine β_2-microglobulin (182), at the same temperature, overnight (12 to 18 hours). The synthetic oligonucleotide 5'–GAGGGTGCTGTCCTGAGACCGAGG–3', complementary to the 5' end of the first exon of $C\alpha$ (183) was 5'–terminally labelled with γ–^{32}P ATP (175) and used as intra–$C\alpha$ probe. The location of this oligonucleotide in relation to the TCR α C region exons is indicated in Figure 5. After hybridization during 14-24 hrs, filters were washed in 2x SSC (0.3M NaCl, 0.03M Na citrate), 0.1% SDS 5' at room temperature, followed by two washes in 0.2X SSC, 0.1% SDS at 60^0C for 15' and a final wash in 0.1X SSC, 0.1% SDS at 60^0C for 10'. To confirm that the same amount of RNA was loaded in each track, the gel was stained with ethidium bromide and/or the filters were hybridized with chicken β-actin cDNA probe (184).

POLYMERASE CHAIN REACTION (PCR)

PCR was performed in a Tyler Thermal Reactor (Tyler Research Instruments Corporation, Edmonton, AB, Canada) according to the method described by Kawasaki (185), after initial reverse transcription of RNA with a specific antisense oligoprimer. Specifically, reverse transcription of 500-1000 ng of total RNA was performed in a final volume of 10 μl using the following reagents in the indicated final concentrations: 50 U of Moloney murine leukemia virus reverse transcriptase (SuperScript® RT, Gibco BRL, Gaithersburg, MD), 50-100 ng of sequence-specific antisense oligonucleotide, 1 mM of each deoxynucleotide, 6 U of placenta RNase inhibitor (RNasin® ribonuclease inhibitor, Promega Corporation, Madison, WI), and PCR buffer (50mM KCl, 20mM Tris pH 8.4, 2.5mM $MgCl_2$, 0.1 mg/ml nuclease-free bovine serum albumin, and 1mM dithiothreitol). The reaction was incubated at 42^0C for 1 hr. After reverse transcription, Taq DNA polymerase (0.5 U) (Gibco BRL), sequence-specific sense oligonucleotide (50-100 ng), PCR buffer to achieve the final concentrations mentioned above, and water were added to the samples and

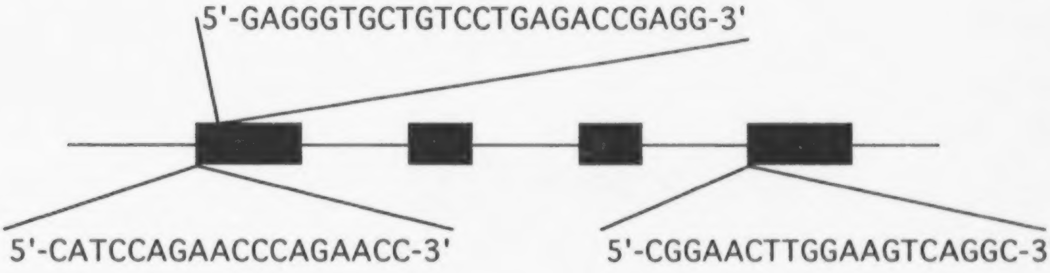
5'-GAGGGTGCTGTCCTGAGACCGAGG-3'
5'-CATCCAGAACCCAGAACC-3'
5'-CGGAACTTGGAAGTCAGGC-3'

Figure 5.- Location of the synthetic oligonucleotides used in these experiments in relation to the Cα gene segment. Map is not draw to scale.

amplified with PCR. The following temperatures were used in each cycle: initial denaturation at 94^0C for 2', and, afterwards at 92^0C for 1', annealing primers at 52^0C for 30", and extension at 72^0C for 1 minute. In the final cycle, samples were kept at 72^0C for 10'. The PCR was stopped after 20 or 40 cycles, depending on the experiment.

For TCR Cα amplification, the sense primer used was 5'-CATCCAGAACCCAGAACC -3', which binds to the 5' end of the first exon of Cα; as anti-sense primer, we used 5'-CGGAACTTGGAAGTCAGGC-3' which is complementary to the 5' end of the fourth exon of Cα (186). Oligonucleotides were synthesized in the DNA synthesis facility at the University of Alberta (Department of Microbiology). The location of these oligonucleotides in relation to the TCR α C region is indicated in Figure 5.

Aliquots from the amplification reactions were run in a 1.5% agarose gel in TBE buffer (0.089M Tris-Borate, 0.089M boric acid, 2mM EDTA).

Specificity of the amplified cDNA was confirmed by hybridization with specific probes, after Southern transfer from the agarose gel on to nylon membranes (175). In each PCR run, a negative control containing primers, buffers, dNTPs, and Taq polymerase but no RNA was included.

SEQUENCING

Amplified products were cloned into the SmaI site of pUC19 and sequenced by the dideoxy method using Sequenase (United States Biochemical Corporation, Cleveland, OH).

MONOCLONAL ANTIBODIES

Mouse monoclonal antibodies (mAb) against the NK-1.1 determinant (PK136; IgG_{2a}) (187), and against a monomorphic determinant of $I\text{-}A^k$ (11-5.2.1.9; IgG_{2b}) (188) were grown in our laboratory from cell lines obtained from American Type Culture Collection (ATCC). A rat anti-mouse Mac-1 α unit (M1/70.15.11.5.HL; IgG_{2b}) was also grown in our laboratory from an ATCC cell line (189). A hamster anti-mouse CD3 ϵ (145-2C11; IgG) (190) was kindly provided by Dr. D.R. Green (La Jolla Institute of Allergy and Immunology, La Jolla, CA). A rat anti-mouse anti-Thy-1.2 mAb (30-H12; IgG_{2b}) was purchased from Becton-Dickinson (191). A murine mAb against mouse leukocyte common antigen (anti-Lyt 5.2; IgG_{2a}) (192) was purchased from Cedarlane Laboratories Ltd. (Hornby, Ontario, Canada). Surface expression of these molecules on cell fractions was performed by flow cytometry after indirect FITC labelling.

A hamster anti-mouse monoclonal antibody (IgG) against an epitope of the constant region of the TCR α chain (H28.710; IgG) was kindly provided by Dr. R. Kubo (National Jewish Center for Immunology and Respiratory Medicine, Denver, CO). This mAb recognizes the TCR α chain when dissociated from CD3 molecules as indicated by the fact that it reacts with TCR α chains obtained from lysates prepared with strong detergents but not with lysates made in digitonin (160).

IRRADIATION AND BONE MARROW RECONSTITUTION EXPERIMENTS

To test the radiosensitivity of the renal cells expressing the truncated TCR α mRNA, individual mice received increasing doses of total body irradiation, from 500 rads to 1500 rads, using a ^{137}Cs γ-irradiator (Gammacell 1000, Nordion International Inc., Kanata, Ont.). Five days after irradiation, mice were sacrificed and kidneys were harvested.

The bone marrow origin of the renal cells expressing the truncated TCR α mRNA was tested by classical bone marrow reconstitution experiments. Bone marrow cells were obtained by flushing femurs of normal mice with RPMI 1640 medium (Gibco BRL). Mice received total body γ-irradiation at dose of 1100 rads immediately after which they received bone marrow cells (23×10^6 i.v. in 0.1 ml of RPMI 1640 per mice) or no cells. Kidneys and spleens were harvested ten days after irradiation-reconstitution.

IMMUNOPEROXIDASE STAINING

The technique for immunoperoxidase staining of cryostat sections has been previously reported (173). Staining with anti-Thy-1.2 mAb and anti-CD3 mAb was used to asses infiltration of renal parenchyma by mature T cells.

IN SITU HYBRIDIZATION

Cryostat sections of kidneys (5 μ) were kept at -20^0C until use. The cDNA probe was labelled to about 0.5×10^8 c.p.m./μg specific activity by nick translation, with ^{35}S labelled dCTP and dATP. Prehybridization and hybridization conditions and post-hybridization washes were performed as described by Gnann et al (193). The slides were incubated in NTB-2 emulsion (Kodak) at 4^0C for 6 days, developed, and counter-stained with haematoxylin and eosin. Eight sections of each tissue on four different slides were evaluated. The TCR $C\alpha$ probe was omitted from the hybridization mix on half of the slides, which served as negative controls.

RNASE PROTECTION ASSAY

RNase protection assays were performed according to an standard procedure (194). Twenty five μg of total RNA were used for each sample. A 450 bp fragment of the TCR α C region cloned into EcoRI-BamHI sites of pBluescript® II KS + (Stratagene Cloning Systems, La Jolla, CA) was used to make antisense and sense single strand RNA probes from the T7 or the T3 promoters respectively. The TCR α C fragment was obtained by two-sided polymerase chain reaction (PCR) of total kidney RNA, was fully sequenced, and confirmed that it contained a correctly spliced TCR α C region, from the 5' end of exon 1 to the 5' end of exon 4 (195). The probes were prepared from the T7 or T3 promoters using 0.5 μg of plasmid template, α-^{32}P CTP (320 μCi), transcription buffer (40mM Tris-HCl pH 8.0, 8mM $MgCl_2$, 2mM spermidine, 50mM NaCl), 10mM dithiothreitol, 40 U of RNasin®, 1mM of ribonucleotides, and 10 U of T7 or T3 RNA polymerase. After incubation for 30 min at 37^0C, RNase-free DNase (10 U) was added and incubated at 37^0C for 30 min. After incubation, 2 μl of 10 mg/ml tRNA and TE were added to a final volume of 50 μl, followed by a phenol-chloroform extraction and two amonium acetate-ethanol precipitations. The pellets were finally dissolved in 100 μl of hybridization buffer (1 volume of 200 mM PIPES pH 6.4, 2M NaCl, 5mM EDTA in 4 volumes of formamide).

Hybridization to RNA was performed at 85^{0}C for 5 min followed by overnight hybridization at 44^{0}C. After hybridization, samples were digested with RNase A (40 μg/μl) in 350 μl of digestion buffer (10mM Tris-Cl pH 7.5, 300mM NaCl, 5mM EDTA) at 30^{0}C for 30 min. After digestion, SDS (10 μl of 20%) and proteinase K (2.5 μl of 20 mg/ml) were added to the samples and incubated at 37^{0}C for 15 min. Samples were extracted with phenol-chloroform, and ethanol precipitated in the presence of tRNA. The pellets were resuspended in 3 μl of loading buffer (80% formamide v/v, 1mM EDTA pH 8.0, 0.1% bromophenol blue, 0.1% xylene cyanol), and run in a 6% polyacrylamide gel (Gibco BRL Ultrapure Marathon Gel Mix, Gibco BRL, Gaithersburg, MD). After drying, the gel was exposed at -70^{0}C for 4 hr.

IMMUNOPRECIPITATION

Protein isolation was performed using the technique reported by Springer with some modifications (196). Tissue homogenates from kidney, liver, and spleen were prepared in 1 ml of TSA solution (0.002M Tris-HCl pH 8.0 at 4^{0}C, 0.14 M NaCl, 0.024% Sodium Azide) using a polytron homogenizer. After pelleting and re-homogenization in 0.5 ml of TSA, tissues were resuspended in five volumes of TSA and an equal amount of lysis buffer (2% Triton X-100, 0.005 M Iodoacetamide, 0.001 M phenylmethylsulfonyl fluoride (PMSF)) and incubated at 4^0C for 1 hour with intermittent mixing. After incubation, samples were spun down (6000 r.p.m. for 10 min) and supernatants were transferred into 0.2 volumes of 5% sodium deoxycholate. After incubation on ice for 10 minutes, the samples were spun at 38000 r.p.m. for 60 minutes at 4^0C, and the supernatants were collected and and run through a double column, immunoaffinity chromatography system. The first column (pre-column) of this system was made of cyanogen bromide-activated Sepharose 4B beads (Pharmacia, LKB Biotechnology, Uppsala, Sweden) coated with rabbit anti-mouse IgG (Cappel Laboratories, West Chester, Pennsylvania). The second column, connected in series to the previous column, was made of H28.710-coated cyanogen bromide-activated Sepharose 4B beads. Columns were washed with 10 column-volumes of a washing buffer containing 0.01 M Tris-HCl (pH 8.0 at 4^0C),).14 M NaCl, 0.025% sodium azide, 0.5% Triton X-100, and 0.5% Sodium deoxycholate, followed by 5 column volumes of Tris buffer (0.05 M Tris, 0.5 M NaCl, 0.1% Triton X-100, pH 8.0), 5 column volumes of Tris buffer (pH 9.0), 5 column volumes of Triethanolamine solution (0.05 M triethanolamine, 0.1% Triton X-100, 0.15 M NaCl, pH 11.5), and 5 column volumes of washing buffer. After applying tissue lysates to the columns, these were washed with washing buffer, the pre-column was disconnected, and the immunoaffinity column was washed with Tris buffer at increasing pHs. To elute the specific antibody-bound antigen, the column was washed with 5 column volumes of triethanolamine solution (pH 11.5) and fractions were collected into tubes containing 0.2 volumes of 1 M Tris-HCl (pH

6.7). The fractions were then concentrated 15 times, and protein determination performed by spectrophotometry at absorbance of 750 nm. Sample aliquots were run in a 8-12% SDS-PAGE gel (Phastsystem®, Pharmacia) and the gel was silver stained with 0.5% silver nitrate followed by 0.015% formaldehyde in 2.5% sodium carbonate as a developer.

CYCLOHEXIMIDE

Cycloheximide was obtained from Sigma (Sigma Chemical Co, St. Louis, MO), dissolved in ethanol and phosphate buffered saline (PBS) at a final concentration of 20 mg/ml and administered i.p. as a single dose of 200 mg/kg. Mice were sacrificed after 6 hours of cycloheximide administration and kidneys were harvested. As controls, mice received the same volume of PBS.

CHAPTER THREE:

RESULTS

DETECTION OF A TRUNCATED TCR α mRNA IN MURINE KIDNEY

Northern blotting of total RNA from different murine tissues with a TCR Cα probe showed, as expected, the presence of a complete transcript for TCR α chain (1.7 kb) only in the spleen (Figure 6). However, we were surprised to find that in the kidney a 1.3-1.4 kb band was consistently detected. A similar size band but much fainter was detected in the brain but not in liver, muscle, or heart.

No complete TCR α transcript was detected in the kidney after 2 day exposures. After longer exposures, a very light band for complete TCR α transcript could be detected in 3 out of 6 experiments. Moreover, no TCR β or δ transcripts were detected when the same northern blots were probed with specific probes for these messages. Specifically, titration experiments to compare the hybridization conditions of the TCR α and TCR β probes showed that, at similar amounts of TCR α mRNA (complete in the spleen and truncated in the kidney), TCR β transcripts could always be detected in the spleen but never in the kidney (Figure 7).

These findings argue that blood contamination was not the explanation for the detection of truncated TCR α mRNA in the kidney because: a)in peripheral lymphocytes, the complete TCR α transcript is the only detected TCR α transcript or is much more abundant than the truncated mRNA when this is detected (66); and b) the complete TCR α mRNA always correlates with the presence of TCR β transcripts.

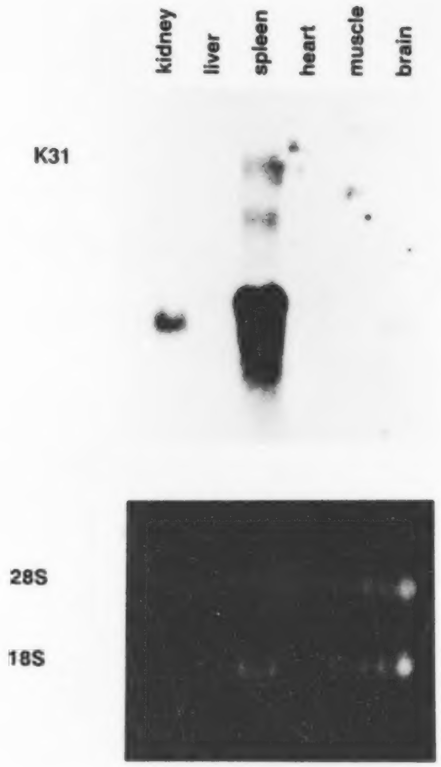

Figure 6.- Tissue distribution of complete (1.7 kb) and truncated (1.3–1.4 kb) TCR α mRNA. Total RNA (30 μg) extracted from different organs (pooled from 5 different mice) was run in a 1.5% agarose, 2.2M formaldehyde gel and transferred in a nitrocellulose filter. The membrane was probed with a TCR Cα cDNA, and exposed for 36 hours. The picture at the bottom shows the gel stained with ethidium bromide, indicating that equal amounts of RNA were loaded in each track. Reproduced with permission (195).

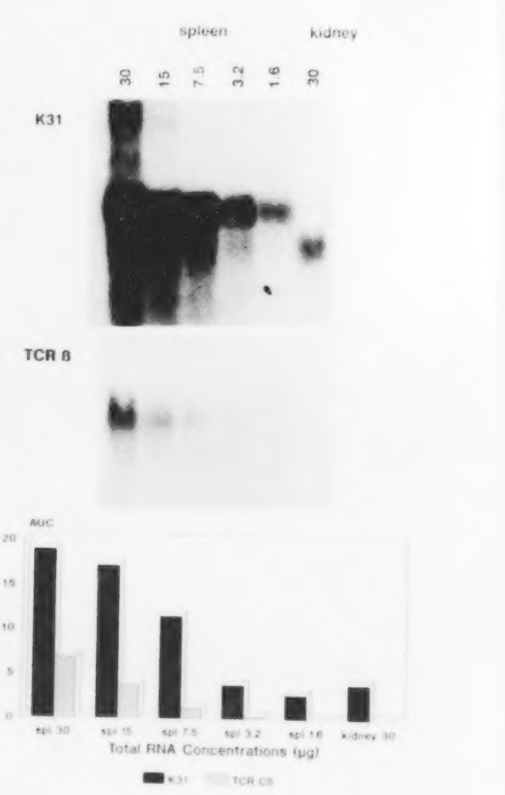

Figure 7.- Titration of hybridization for the TCR α and TCR β probes. Different amounts of spleen total RNA and 30 µg of kidney total RNA were northern blotted and probed for TCR α and TCR β, under identical conditions. Results of these hybridizations are expressed in densitometric readings and AUC in the bottom graph.

THE CELLS EXPRESSING THE TRUNCATED TCR α mRNA ARE NOT T CELLS OR NK CELLS

To localize the cell type expressing the truncated TCR α message, we performed in situ hybridization of murine kidney cryostat sections with a TCR α cDNA. Strongly positive cells (2 to 5 per 0.2 cm^2) were detected underlying the tubular epithelium, in the renal interstitium-subepithelial space (Figure 8).

Next, we studied the expression of the truncated TCR α mRNA in renal cortex and medulla. Northern blots using total RNA extracted from cortex and medulla showed that the truncated TCR α mRNA was present in both cortex and medulla, but was relatively more abundant in the medulla (Figure 9).

To investigate whether the cell type expressing this truncated message was a T cell or an NK cell, we enriched renal interstitial cells according to a previously reported method (174) which uses collagenase digestion, followed by Percoll gradient centrifugation. This procedure is intended to deplete the final cell suspension of glomeruli and tubule cell clumps, but a certain amount of contamination by these cells is invariably present. Initial estimates of contamination by tubule epithelial cells using immunoperoxidase staining for cytokeratin indicates that epithelial cells constitute up to 15-20% in fraction <40% (see below). Nevertheless, such fractionation permits correlations between surface markers and relative content of specific mRNAs.

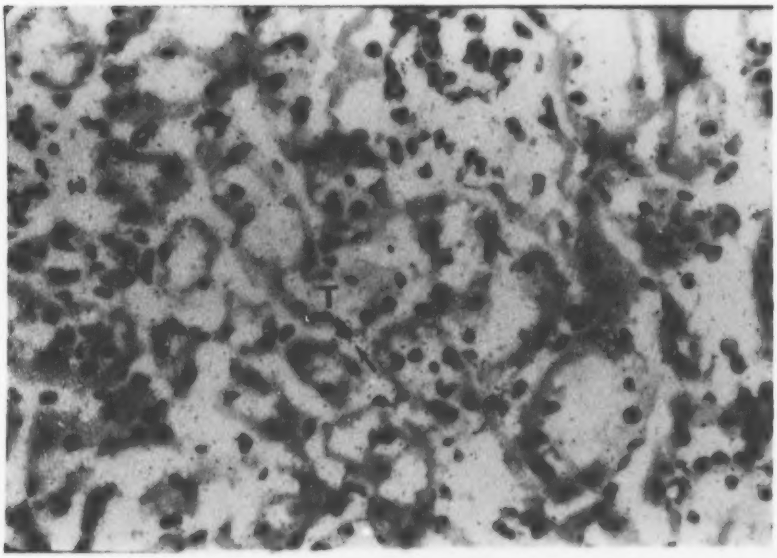

Figure 8.- In situ hybridization of kidney sections for TCR Cα, showing a positive cell in the renal interstitial space (arrow). In situ hybridization was performed using a TCR α C region cDNA probe (see technique in text). T: renal tubule lumen. Reproduced with permission (195).

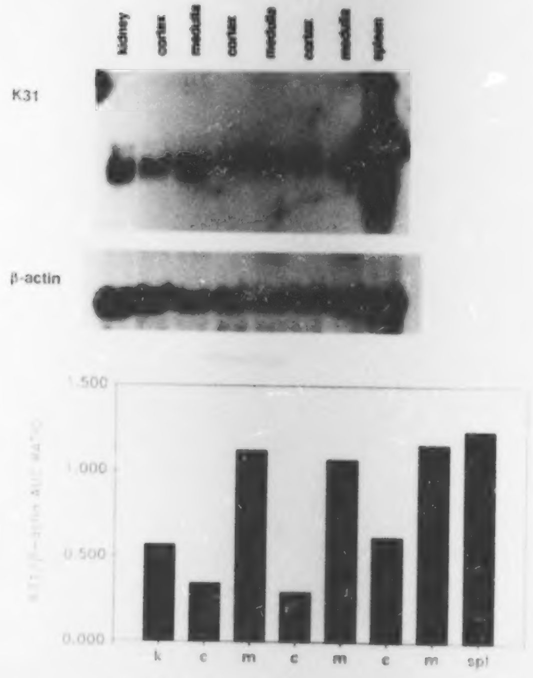

Figure 9.- The cells expressing the truncated TCR α mRNA are more abundant in the renal medulla. Kidneys from three individual mice were harvested and, after dissection of cortex and medulla, total RNA was extracted from each preparation, northern blotted (30 μg), and probed for TCR α and β-actin. The graph at the bottom shows the ratio of densitometric readings of TCR α over ß-actin. (k: whole kidney, c: renal cortex, m: renal medulla, spl: spleen). Reproduced with permission (195).

Northern blot analysis was performed from total RNA extracted from 4 different renal cell fractions obtained by Percoll gradient centrifugation (Percoll gradients: <40%, 40-50%, 50-70%, and 70-100%). The truncated form was present in fraction <40% (Figure 10). No transcripts for complete TCR α, TCR β or TCR δ chains were detected in this fraction. In fraction 40-50% and 50-70%, the complete form of TCR α mRNA was detected (Figure 10). Expression of T cell markers (CD3 and Thy-1) and an NK cell marker (NK-1.1) was then assessed for each fraction (Table I). CD3 and Thy-1 expression correlated with the presence of complete TCR α mRNA but not with the presence of the truncated form. For CD3 expression, the percentage of positive cells were: 0% in fraction <40%, 4% in fraction 40-50%, 44% in fraction 50-70%, and 0% in fraction 70-100%. No Thy-1 expression was detected in fractions <40%, and 70-100%; 1% of cells of fraction 40-50% and 42% of the cells in fraction 50-70% were positive for Thy-1 expression. This finding suggests that the cells expressing the truncated TCR α mRNA are not mature T cells expressing a functional TCR/CD3 complex on the surface (2). In addition, no significant expression of NK-1.1 was detected in fraction <40%, suggesting that the cells expressing the truncated TCR α mRNA are not NK cells. Northern blotting with a class II probe showed a relatively low level of expression of class II mRNA in fraction <40% in comparison to fractions 40-50% (predominantly macrophages) and 50-70% (which contains T and B cells).

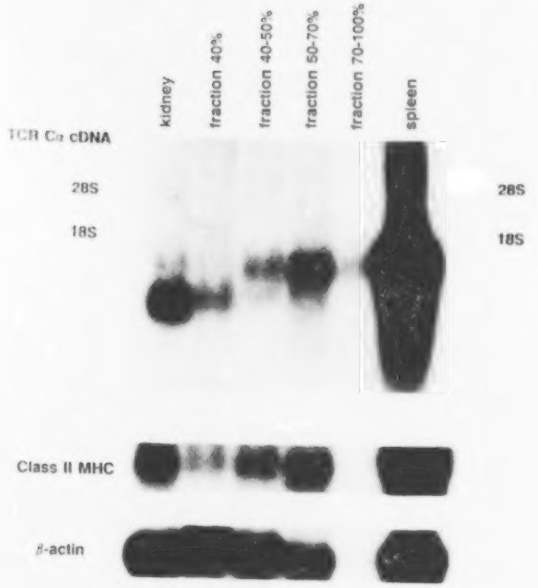
kidney
fraction 40%
fraction 40-50%
fraction 50-70%
fraction 70-100%
spleen
TCR Cα cDNA
28S
18S
28S
18S
Class II MHC
β-actin

Figure 10.- The truncated form of TCR α mRNA is expressed by cells contained in Percoll fraction <40%. Renal cell suspensions enriched for interstitial cells were fractionated through Percoll gradient centrifugation. Total RNA (5.5 μg) was extracted from each fraction (Percoll gradients: <40%, 40-50%, 50-70%, and 70-100%), was northern blotted and probed for TCR α, class II MHC (I–A^k), and β-actin using the probes reported in the text. Reproduced with permission (195).

Further characterization of the phenotype of the cells expressing the truncated TCR α mRNA is currently limited by the absence of techniques for isolating pure cell suspensions from renal tissue in viable state. To date, our efforts to obtain enough RNA after positive and negative selection of fraction <40% with monoclonal antibodies to study pure candidate cell populations have been hampered by low cell viability. A more detailed analysis of the phenotype of the cells isolated in each fraction is presented in Table I. However, it should be born in mind that the cell populations in each fraction are heterogenous.

THE TRUNCATED TCR α mRNA IS EXPRESSED BY RADIOSENSITIVE CELLS OF BONE MARROW ORIGIN IN A THYMUS-INDEPENDENT FASHION

To investigate the radiosensitivity of the cells expressing the truncated TCR α mRNA, we exposed mice (n = 3 to 6) to whole body irradiation with ^{137}Cesium at doses of 500, 750, 1000, 1500, and 2000 rads, and sacrificed after 5 days. Mice receiving 2000 rads did not tolerate the procedure and very few (up to 45%) survived after 5 days. Thus this group was not included in further experiments. As shown in Figure 11, the cells expressing the truncated TCR α mRNA are radiosensitive, as shown by a dose-dependent reduction of the steady state truncated TCR α mRNA with increasing doses of radiation, and with more than 85% reduction of this message 5 days after receiving 1500 rads.

TABLE I

Phenotype of the different renal interstitial cell fractions

	<40%	40-50%	50-70%	70-100%
Cells/Kidney (x10^5)*	6.9±5.4	2.9±1.8	5.3±3.4	<0.05**
Marker				
Thy-1	0	1	42	0
CD3	0	4	44	0
Class II MHC	3	8	55	0
CD45	16	22	81	0
Mac-1	7	6	3	0
NK-1.1	<1	0	0	0

Renal interstitial cells were separated by enzymatic digestion And Percoll gradient centrifugation, after sieving of 14 to 20 kidneys from CBA/J mice. Five hundred thousand cells were stained with the appropriate mAb followed by anti-rat or anti-mouse FITC-labelled $F(ab')_2$ fragments. For each mAb, a control group stained only with FITC-labelled $F(ab')_2$ fragments was included in each fraction. Cells were analyzed by flow cytometry. Numbers for each surface marker indicate % of positive cells in 10,000 cells. *Mean±SD from 6 experiments. Cells were stained with Trypan Blue and live cells were counted. Glomeruli and tubule cell clumps were not counted. **Only cells other than red blood cells were counted.

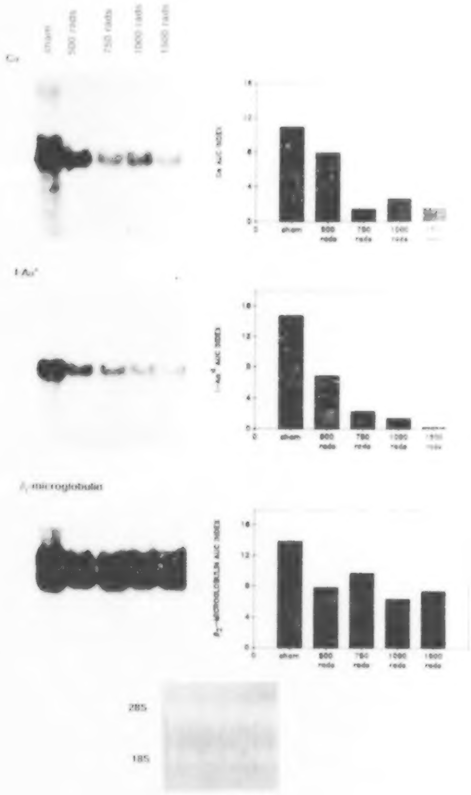

Figure 11- Effect of radiation on the expression of truncated TCR α mRNA by renal interstitial cells. Mice were irradiated with ^{137}Cs at the indicated doses, and sacrificed after five days. Pooled total RNA extracted from kidneys (n = 3) was northern blotted and probed for TCR α, class II and β_2-microglobulin using the probes noted in the text. The optical density of each band in the Northern blot was measured and expressed as area under the curve (AUC). Staining of the gel with ethidium bromide confirmed that similar amounts of RNA were loaded in each track.

Parallel reduction of the levels of class II MHC mRNA in the kidneys of these mice was observed; however, no dose-dependent decrease of β_2-microglobulin mRNA was detected, suggesting that class I is expressed by radioresistant cells, like epithelial cells (Figure 11). The radiosensitivity of the cells expressing the truncated TCR α mRNA suggested that these cells were bone marrow dependent. To confirm this hypothesis, lethally irradiated mice were reconstituted with bone marrow cells and the expression of the truncated TCR α mRNA in the kidney was tested ten days later. As shown in Figure 12, the expression of the truncated TCR α mRNA in the kidney is restored with bone marrow reconstitution after total body irradiation, in contrast to the marked reduction of steady state levels for this message in the kidney of irradiated, non-reconstituted mice. From this experiment we concluded that the cells expressing the truncated TCR α mRNA are bone marrow dependent. This finding prompted us to test the presence of this transcript in bone marrow. Northern blots of total RNA extracted from bone marrow showed that the truncated transcript was not expressed in the bone marrow (Figure 13). Therefore, the expression of the truncated TCR α mRNA is likely to be dependent on the renal microenvironment because the cells expressing the truncated TCR α mRNA are probably of bone marrow origin but do not express this transcript in the bone marrow.

Although the fractionation experiments presented above were consistent with the hypothesis that the cells expressing the truncated TCR α mRNA were not mature, conventional T cells, we decided to test the hypothesis that these cells

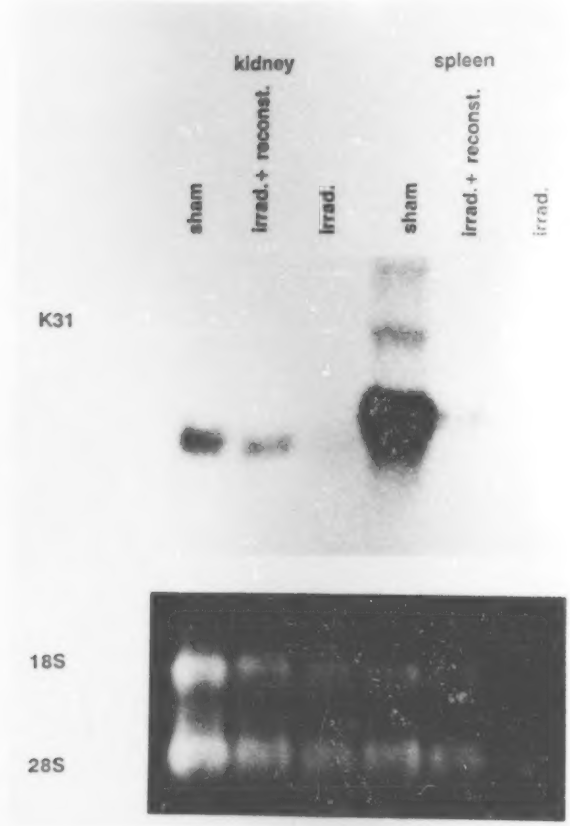

Figure 12.- The renal cells expressing the truncated TCR α mRNA are bone marrow-dependent. Total RNA from kidney and spleen of individual control mice, irradited mice reconstituted with bone marrow cells, and irradiated mice not reconstituted was blotted and probed for TCR α. Eleven hundred rads of total body irradiation was admininistered. Reconstitution was performed with i.v. injection of syngeneic bone marrow cells (23×10^6 cells per mouse). Organs were harvested 10 days after irradiation/reconstitution. The picture of the ethidium bromide stained gel is shown at the bottom. Note that the low amount of RNA in the spleen of the irradiated, non-reconstituted mouse is due to the marked atrophy of the spleen after lethal irradiation.

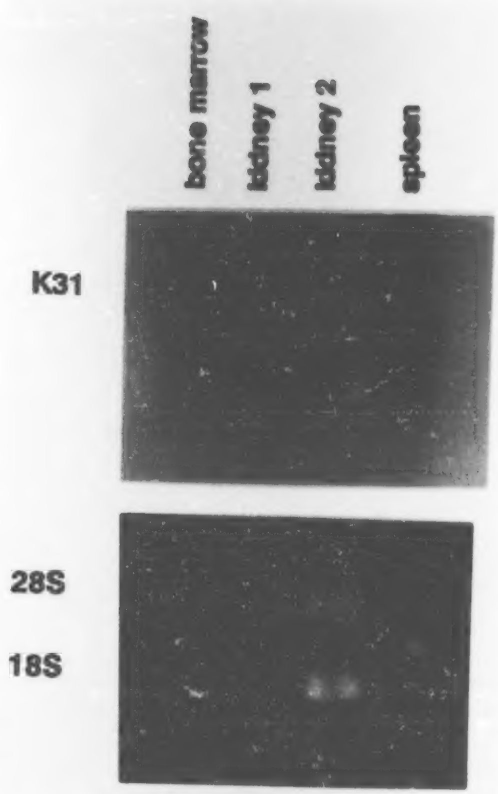
bone marrow
kidney 1
kidney 2
spleen
K31
28S
18S

Figure 13.- The truncated TCR α mRNA is not detected in the bone marrow. Northern blot of total RNA (30 μg) from bone marrow, two kidneys, and spleen of normal (non-manipulated) CBA/J mice probed for TCR α. Ethidium bromide staining of the gel (shown at the bottom) indicates that similar amounts of RNA were loaded in each track. Reproduced with permission (195).

were thymus-derived. Nude mice have only rudimentary thymuses, and virtually no T cells when they are young. Northern blots of kidney and spleen total RNA showed that there was little or no complete TCR α transcript in the spleens of nude mice but the truncated TCR α mRNA could be detected in the kidneys of nude mice in levels similar to the normal controls (Figure 14), implying that the presence of this message is not dependent on normal thymic function. Because the nude mice used for these experiments were young (8 weeks old), the results could not be explained by possible peripheral T cell contamination.

THE EXPRESSION OF THE TRUNCATED TCR α mRNA DOES NOT REQUIRE AN INTACT RECOMBINASE ACTIVITY

Successful rearrangement of immunoglobulin and TCR genes requires the presence of a common, functional recombinase activity. Mice with the SCID mutation are severely deficient in T and B cells. This disorder has been attributed to a defect in the recombinase activity, which leads to aberrant gene rearrangements and the lack of functional Ig and TCR gene rearrangements (197). We tested SCID mice for the expression of truncated TCR α mRNA to determine whether the expression of this transcript was dependent on functional recombinase activity. Northern blots of total RNA extracted from kidneys and spleens of mice with the SCID mutation and normal controls showed that the truncated TCR α mRNA in the kidney was not affected by the SCID mutation (Figure 15). In contrast, the complete TCR α mRNA was virtually absent in the spleens of SCID

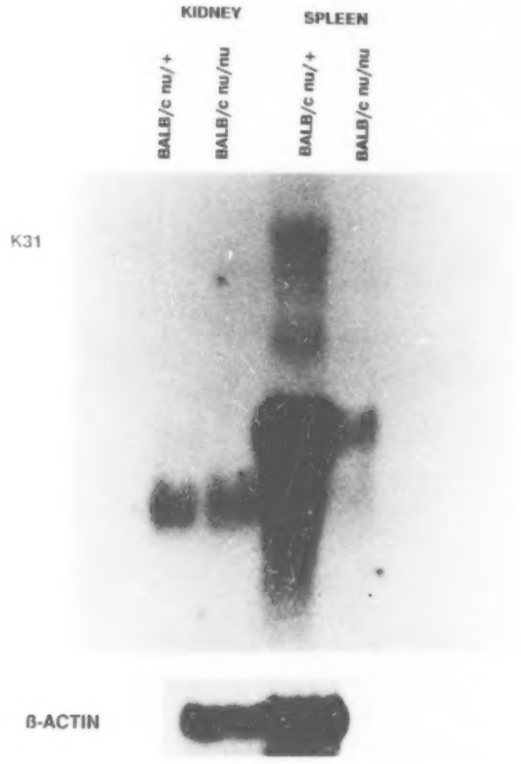
KIDNEY
SPLEEN
BALB/c nu/+
BALB/c nu/nu
BALB/c nu/+
BALB/c nu/nu
K31
ß-ACTIN

Figure 14.- The population expressing the truncated form of TCR α mRNA is thymus-independent. Northern blot of total RNA (30 μg) from kidneys and spleens of nude mice (BALB/c nu/nu) and normal littermates (BALB/c nu/+) probed with TCR Cα and β-actin cDNA probes. Reproduced with permission (195).

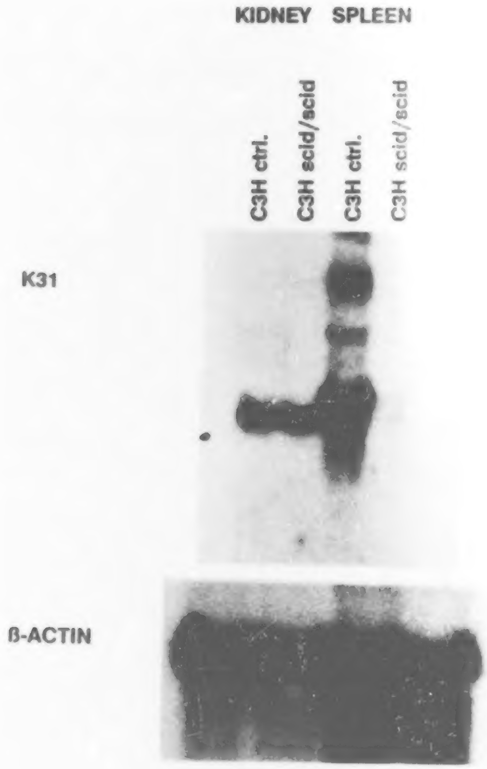
KIDNEY SPLEEN
C3H ctrl.
C3H scid/scid
C3H ctrl.
C3H scid/scid
K31
ß-ACTIN

Figure 15.- The expression of the truncated TCR α mRNA is not affected by the SCID mutation. Northern blot of total RNA (30 μg) from kidneys and spleens of C3H mice with the SCID mutation and normal C3H mice probed with TCR Cα and β-actin cDNA probes. Reproduced with permission (195).

mice. We conclude that the truncated TCR α mRNA detected in murine kidney is not due to T cell contamination, does not require a competent recombinase activity, and is likely a germline transcript.

THE TRUNCATED TCR α mRNA CONTAINS Cα

To confirm that this band corresponded to a truncated form of TCR α mRNA, the following experiments were performed. First, the same northern blots were hybridized with a terminally-radiolabelled 24 oligonucleotide probe complementary to the first exon of Cα. Although the degree of cross-hybridization of this synthetic oligonucleotide to the 28S ribosomal RNA is considerable, the results showed the same banding pattern for the complete and truncated TCR α mRNAs as the one detected with the TCR Cα probe (Figure 16). From this experiment we concluded that the band detected by northern blotting most likely contained a Cα segment. Second, we tried to amplify the Cα region from total RNA extracted from kidney with sense and antisense primers specific for the 5' ends of the first and the fourth exons, respectively. The expected size of the fragment flanked by these two primers is 450 nucleotides, considering only the exon sequences. Amplification using PCR showed, in addition to the expected 450 nucleotide fragment, the presence of at least three bands of about 220, 600, and 700 nucleotides from kidney total RNA (Figure 17). Similar bands were detected after amplification of spleen total RNA. We failed to amplify TCR Cα mRNA from liver total RNA, suggesting that these results were likely not due to amplification of

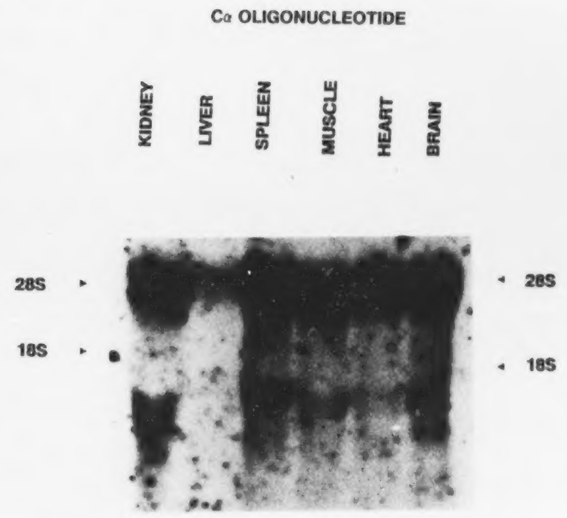
Cα OLIGONUCLEOTIDE
KIDNEY
LIVER
SPLEEN
MUSCLE
HEART
BRAIN
28S
18S
28S
18S

Figure 16.- Northern blot of total RNA from different organs of normal mice probed with a synthetic oligonucleotide complementary to the TCR α C region (5' end of the first exon). The sequence of this oligonucleotide is 5'-GAGGGTGCTGTCCTGAGACCGAGG-3'). Hybridization conditions are specified in the Material and Methods chapter.

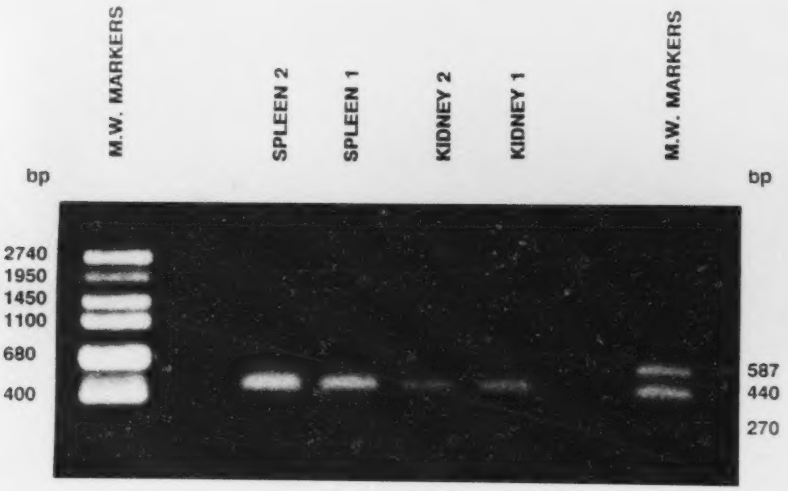
M.W. MARKERS
SPLEEN 2
SPLEEN 1
KIDNEY 2
KIDNEY 1
M.W. MARKERS
bp
bp
2740
1950
1450
1100
680
400
587
440
270

Figure 17.- Amplification of TCR Cα mRNA by PCR. One hundred ng of total RNA extracted from spleen and kidney were used for amplification with 40 cycles of PCR (denaturation, annealing, and extension temperatures are specified in the text). The amplified products were run in a 1% agarose gel and stained with ethidium bromide. Specificity of these products was confirmed by Southern blotting using a TCR Cα cDNA probe (data not shown). Reproduced with permission (195).

RNA from circulating T lymphocytes. Third, we sequenced the 450 bp amplified product from total kidney RNA. The sequence obtained from two clones containing this fragment confirmed that it was the product of correctly spliced Cα1, Cα2, Cα3 and Cα4 exons of Cα, flanked by the primers used for amplification (Figure 18). In addition, the same truncated TCR α transcript could be detected in kidney and brain when northern blots were probed with this fragment.

THE TRUNCATED TCR α mRNA CONTAINS A CORRECTLY SPLICED TCR α C REGION

From the previous experiments we concluded that normal mouse kidney expresses a truncated TCR α mRNA that it is not associated with expression of other TCR genes, is radiosensitive, thymus-independent and recombinase activity-independent. Although the specificity of the transcript was strongly supported by probing northern blots with specific TCR α oligonucleotides, amplification of the TCR α C region from murine kidney with two-sided PCR, cloning of these amplified products, and sequencing, in order to fully establish that the transcript expressed in the kidney contained the TCR α C region, we performed an RNase protection assay using RNA from kidneys and spleens of nude and scid mice. In these animal, we had shown that the expression of complete TCR α mRNA is severely diminished or abolished and, in the kidney, the truncated transcript is practically the only expressed. Therefore, any protected fragment in the kidney of these mice comes from the expression of truncated TCR

+1 +44
K31 C ATC CAG AAC CCA GAA CCT GCT GTG TAC CAG TTA AAA GAT CCT
Cα C ATC CAG AAC CCA GAA CCT GCT GTG TAC CAG TTA AAA GAT CCT

+45 +89
CGG TCT CAG GAC AGC ACC CTC TGC CTG TTC ACC GAC TTT GAC TCC
CGG TCT CAG GAC AGC ACC CTC TGC CTG TTC ACC GAC TTT GAC TCC

+90 +134
CAA ATC AAT GTG CCG AAA ACC ATG GAA TCT GGA ACn TTC ATC ACT
CAA ATC AAT GTG CCG AAA ACC ATG GAA TCT GGA ACG TTC ATC ACT

+135 +179
GAC AAA ACT GTG CTG GAC ATG AAA GCT ATG GAT TCC AAG AGC AAT
GAC AAA ACT GTG CTG GAC ATG AAA GCT ATG GAT TCC AAG AGC AAT

+180 +224
GGG GCC ATT GCC TGG AGC AAC CAG ACA AGC TTC ACC TGC CAA GAT
GGG GCC ATT GCC TGG AGC AAC CAG ACA AGC TTC ACC TGC CAA GAT

+225 +269
ATC TTC AAA GAG ACC AAC GCC ACC TAC CCC AGT TCA **GA**C GTT CCC
ATC TTC AAA GAG ACC AAC GCC ACC TAC CCC AGT TCA **GA**C GTT CCC

+270 +314
TGT GAT GCC ACG TTG ACC GAG AAA AGC TTT GAA ACA **GA**T ATG AAC
TGT GAT GCC ACG TTG ACC GAG AAA AGC TTT GAA ACA **GA**T ATG AAC

+315 +359
CTA AAC TTT CAA AAC CTG TCA GTT ATG GGA CTC CGA ATC CTC CTG
CTA AAC TTT CAA AAC CTG TCA GTT ATG GGA CTC CGA ATC CTC CTG

+360 +404
CTG AAA GTA GCG GGA TTT AAC CTG CTC ATG ACG CTG AGG CTG TGG
CTG AAA GTA GCG GGA TTT AAC CTG CTC ATG ACG CTG AGG CTG TGG

+405 +449
TCC AGT TGA **GG**T CTG CAA GAC TGA CAG AGC CTG ACT TCC AAG TTC
TCC AGT TGA **GG**T CTG CAA GAC TGA CAG AGC CTG ACT TCC AAG TTC

+450
CG
CG

Figure 18.- Sequence of the products obtained after PCR amplification of kidney total RNA with specific TCR α C region oligoprimers. After PCR, amplified products were cloned into the SmaI site of pUC 19 and sequenced by the dideoxy method (see text). The sequence of one of the clones (K31) is compared with the sequence of the TCR α C region (Cα) from references 183 and 186. The underlined bases are the sequence of the oligoprimers used for amplification. The bases in bold indicate the splicing donor and acceptor sites of the TCR α C exons.

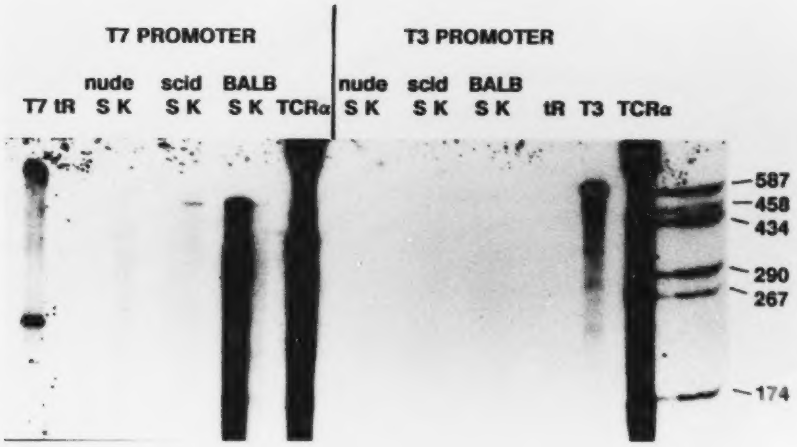
T7 PROMOTER
T3 PROMOTER
nude
scid
BALB
nude
scid
BALB
T7 tR S K S K S K TCRα S K S K S K tR T3 TCRα
587
458
434
290
267
174

Figure 19.- RNase protection assay of total kidney and spleen RNA from normal, nude, and scid mice. The antisense, single-strand probe resulted from T7-directed transcription producing a 534 nt band up to the EcoRI site. The single-strand probe resulting from transcription directed by the T3 promoter up to the BamHI site corresponds to a 536 nt band which was sense to the TCR α C strand and did not hybridize with any message in the RNA samples. Molecular weight markers are shown on the right. Note the presence of a 450 bp protected band in the kidneys of normal, nude, and scid mice, and in the spleen of normal mice. The TCRα lane shows protection of the TCR α C region cDNA probe with the TCR α C region transcript.

α mRNA and not from the complete TCR α mRNA.

As shown in Figure 19, the antisense, single-strand RNA probe protected a fragment of approximately 450 nt in the kidney of nude and scid mice as well as in kidney and spleen of normal mice. However, in the spleen of nude and scid mice, the amount of protected fragment was almost non-existent. The size of this protected fragment is consistent with the 450 nt TCR α C sequence of the probe. The band in the T7 and T3 is around 535 nt as the RNA probe also contains a portion of the polycloning site resulting from the space between the promoter and the starting nt of the TCR α fragment.

Different attempts to characterize the 5' end of the truncated TCR α mRNA have thus far been unsuccessful. These include anchored PCR (198), construction of cDNA libraries from total kidney RNA as well as poly-A^+ RNA, and direct RNA sequencing. On the basis of these negative results, we hypothesized that the TCR α C region could have the ability to form multiple secondary structures which would minimize the efficiency of cDNA synthesis, resulting in a lack of representation of this transcript in the cDNA libraries. Close analysis of the TCR α C region shows that, as suggested, there are at least two major potential secondary structures with 70 to 80% base pair complementarity (including non-Watson and Crick bp). These secondary structures are located 4 bases downstream of the beginning of the TCR α C region and 210 bases downstream of the TCR α C region (Figure 20).

```
      C  C
      A  T
    A      G
      G  C
      A  T
      C  G                                         A
    C      T                                    T      T
      C  G                                        A  T
      A  T                                        G  C
    A      A                                      A  T
      G  C                                        A  T
      A  C                                        C  C
      C  A                                        C  A
      C  G                                      G      A
    T      T                                      T  A
      A  T                                        C  G
      C  A                                        C  A
5' ---A    AAAGATCCTCGGTCTCAGG//GCAACCAGACAAGCTTCA    GA---3'
```

Figure 20.- Two potential secondary structures can be formed in the TCR α C region. The sequence of the TCR α C region was analyzed for potential internal secondary structures. Watson-Crick and non-Watson-Crick base pairing were considered.

THE TRUNCATED TCR α mRNA IS POLYADENYLATED AND CAN BE DETECTED IN THE CYTOPLASMIC FRACTION

Next, we studied the processing pathway of the truncated TCR α mRNA. From the data presented above, we already concluded that this transcript was correctly spliced. The next two steps in processing of RNA before translation are polyadenylation and cytoplasmic transport.

In order to test whether the truncated TCR α mRNA was polyadenylated, poly-A^+ RNA was isolated through oligo-(dT)-cellulose column and northern blotted. As shown in Figure 21, the truncated TCR α mRNA was highly enriched in the poly-A^+ fraction of RNA, being detectable in significant amounts in as low as 1.6 μg of poly-A^+ RNA.

Next, we tested if the truncated TCR α mRNA could be detected in the cytoplasm. After modification of a technique for isolating cytoplasmic RNA from solid organs (see Material and Methods), repeated experiments showed that the truncated TCR α mRNA could be detected in the cytoplasmic fraction of RNA (Figure 22).

The evidence indicating that the truncated TCR α mRNA followed a normal processing pathway strongly supported the hypothesis that this transcript could be translated into a protein.

DETECTION OF A TRUNCATED TCR α PROTEIN IN NORMAL KIDNEY

To demonstrate that the truncated TCR α mRNA was translated in to a

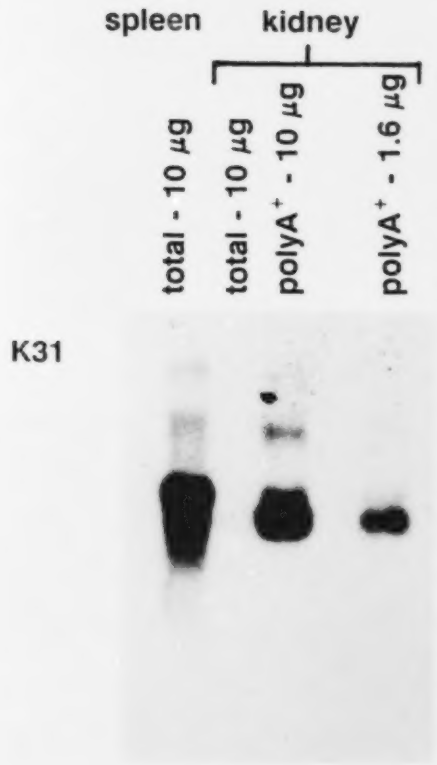
spleen
kidney
total - 10 μg
total - 10 μg
polyA⁺ - 10 μg
polyA⁺ - 1.6 μg
K31

Figure 21.- The truncated TCR α mRNA is polyadenylated. Northern blot of total and poly-A$^+$ RNA from normal kidneys and spleen probed for TCR α. Poly-A$^+$ RNA was extracted from total RNA by oligo-dT cellulose column chromatography.

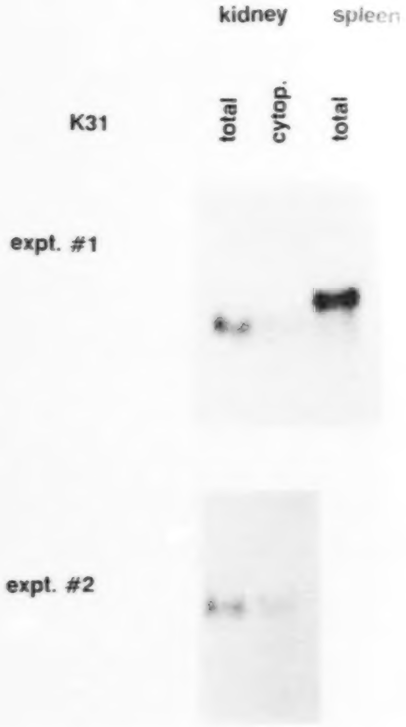
kidney
spleen
K31
total
cytop.
total
expt. #1
expt. #2

Figure 22.- The truncated TCR α mRNA can be detected in the cytoplasm. Total and cytoplasmic RNA were isolated (see Material and Methods), northern blotted, and probed for TCR α. The results of two different experiments are shown.

protein, we used a mAb that is directed against an epitope of the TCR α chain and only recognizes this epitope when the TCR α chain is not associated with CD3 molecules (160).

Using this mAb for immunoprecipitation and SDS-PAGE under reducing conditions, we were able to detect the expected 45 kd band corresponding to complete TCR α chain only in the spleen. In addition, a band of about 30 kd was detected in spleen and kidney, but not in liver (Figure 23). A lower band of about 26 kd was also detected in spleen and kidney samples but its size is compatible with the size of light chains and may be the result of light chains of the mAb eluted with the protein. Although this result is not conclusive, the 30 kd band is compatible with the expected size of a J-C transcript (see Appendix). However, it should be noted that the non-glycosylated TCR α chain is of similar size.

THE EFFECT OF CYCLOHEXIMIDE ON THE EXPRESSION OF TRUNCATED TCR α mRNA IN THE KIDNEY

It has been reported recently that the expression of a protein resulting from reading frame 2 of a partially rearranged D-J H chain genes of Ig controls Ig H chain gene rearrangement by stopping V to DJ rearrangement (199). This event requires the transmembrane expression of the protein, as transgenic mice for a soluble form of the protein do not go through this regulatory mechanism. Based on this result, we hypothesized that the expression of the truncated TCR α protein could be involved in stopping rearrangement of TCR α gene in the kidney. To test

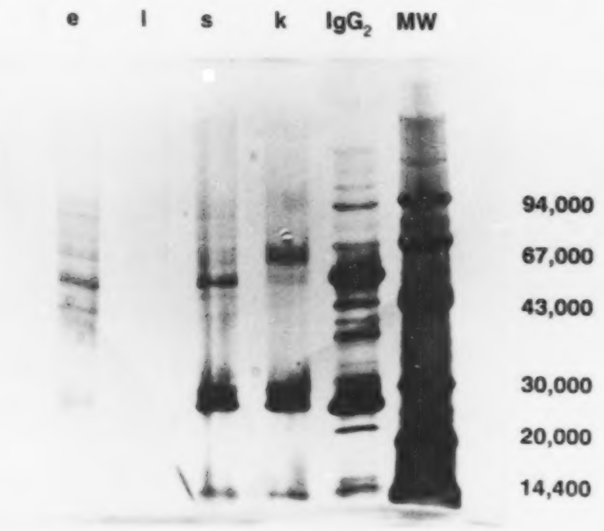

Figure 23.- Immunoprecipitation of complete and truncated TCR α polypeptides with mAb anti-TCR α. Tissue homogenates from kidney, spleen, and liver were applied to an immunoaffinity column coated with anti-TCR α mAb (H28.710). Antigens were eluted and run in an SDS-PAGE 8-12% gel under reducing conditions. The gel was silver staining. Note the 45 kd band present only in the spleen which corresponds to the complete TCR α chain. In addition, two bands in the range of 26 to 30 kd were detected in kidney and spleen homogenates (the lower band of about 26 kd in spleen and kidney samples may correspond to eluted light chains from the Ab column; see text).

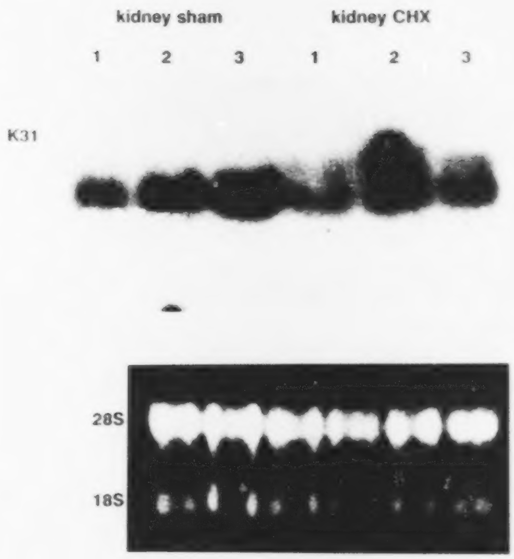

Figure 24.- Effects of in vivo administration of cycloheximide on the expression of truncated TCR α mRNA in normal mouse kidney. Cycloheximide (200 mg/Kg) or PBS were administered i.p. and mice were sacrificed six hours later. Total RNA from kidneys was extracted, northern blotted, and probed for TCR α. The numbers "1, 2, 3" indicate three different mice for each treatment group.

this hypothesis we blocked translation of the truncated TCR α mRNA in vivo by administering cycloheximide.

Six hours after administration of cycloheximide, normal mouse kidneys express truncated TCR α mRNA in slightly lower amounts than in control mouse kidneys. However, in cycloheximide-treated mice, a 1.7 kb band consistently appears at much higher levels that the truncated TCR α mRNA (Figure 24). The size of this band is compatible with the size of complete TCR α mRNA. However, the appearance of this transcript is not associated with appearance of TCR β mRNA (by northern blotting) or with histological evidence of T cell infiltration in the kidney, as indicated by the lack of $CD3^+$ or Thy-1^+ infiltrating cells in kidney sections. As cycloheximide increases transcription of TCR β genes but not of TCR α genes (140), we believe that the appearance of the 1.7 kb mRNA is the result of TCR α gene rearrangement. Confirmation of this hypothesis will require studies of TCR α DNA rearrangement in pure cell populations after CHX treatment. The possibility of alternative effects of cycloheximide on TCR α mRNA half life are considered in the discussion section.

CHAPTER FOUR:

DISCUSSION

SUMMARY OF RESULTS

The results presented in this thesis indicate that among the cell populations of mouse kidney there is invariably a population characterized by relatively abundant expression of a truncated form of TCR α mRNA. The truncated TCR α transcript is not associated with detectable transcription of other TCR genes or with expression of CD3 or Thy-1, arguing that this cell population is not a mature T cell population and does not express functional TCR. This transcript is enriched in the Percoll fraction containing large interstitial cells and is more abundant in the renal medulla. Expression of the truncated TCR α mRNA is abolished by radiation and restored by bone marrow reconstitution indicating that the transcript is expressed in marrow-dependent cells. However, such cells are not thymus-dependent, because the truncated TCR α mRNA is expressed in nude mice. In addition, the expression of the truncated TCR α mRNA is not affected by the scid mutation. Together, these results demonstrate that the truncated TCR α transcript is expressed by non-T cells of probable bone marrow origin. However, the transcript is not expressed in the bone marrow, implying that expression of this transcript is dependent on the renal environment.

Analysis of the molecular biology of the transcript indicates that the transcript contains a correctly spliced TCR α C region, with conservation of the exon coding for the transmembrane domain. In addition, the transcript is polyadenylated, and can be detected in preparations of cytoplasmic RNA. Together, these results show that the truncated TCR α mRNA is normally processed and is likely to be translatable. Immunoprecipitation experiments using a mAb against an epitope of the TCR α C region are compatible with the hypothesis that the truncated transcript is translated into a protein of molecular weight around 30 kd, in contrast to the complete, functional TCR α chain whose molecular weight is 45 kd.

Our results indicate that expression of the truncated TCR α mRNA in normal kidney is affected by the administration of cycloheximide. Administration of cycloheximide is associated with slight decrease of truncated TCR α mRNA steady state levels and, more significantly, with the appearance of a 1.7 kd band whose size is consistent with the size of complete TCR α mRNA. The presence of this larger band hybridizing with the TCR α probe in the kidney after cycloheximide administration is not associated with expression of other TCR genes or with any histological evidence of T cell infiltration. Our interpretation of this result is that cycloheximide inhibits the translation of the truncated TCR α mRNA and that, blocking the expression of the resulting protein may result in full rearrangement of the TCR α gene. Further work is needed to demonstrate this hypothesis is true.

The significance of these results will be discussed from two different perspectives. First, the findings reported in this thesis will be discussed in the context of the truncated TCR α mRNA as an indicator of a particular set of renal interstitial cells. Second, we will discuss the potential function of the truncated TCR α mRNA, and, in particular, its role in TCR α gene rearrangement.

THE TRUNCATED TCR α mRNA AS AN INDICATOR OF A PARTICULAR RENAL INTERSTITIAL CELL POPULATION

The expression of TCR genes is predominantly T cell specific (3, 16, 18, 19, 40, 41, 86, 99). However, transcripts from TCR α genes in germ line configuration or from unsuccessfully rearranged genes are detected during T lymphocyte development in the thymus as well as in B lymphocytes and some CD3 positive cell lines with NK activity (40, 86).

Two main possibilities should be discussed when considering the cellular origin of the truncated TCR α mRNA. The cells expressing the truncated TCR α mRNA may be blood cells contaminating the RNA preparations from normal mouse kidney. Alternatively, the cells expressing the truncated TCR α mRNA may be resident cells in the kidney.

The role of T cells from the blood contaminating the kidney preparations is excluded by the lack of detectable TCR β or δ mRNA and by the absence of complete TCR α message in northern blots from kidneys, as these findings are constant in PBL (66). In addition, the presence of these cells in athymic mice, their low buoyant density, and the lack of concomitant expression of Thy-1 and CD3, all argue that these cells are not T cells, either from blood contamination or resident in the kidney.

The possibility that the cells expressing the truncated TCR α mRNA could be large granular lymphocytes (LGL) must be given serious consideration. This would imply that murine kidney contains a relatively constant population of LGL cells, and that LGL cells are present in the kidney in relatively high number in comparison to other organs (200). However, to my knowledge, no study has reported the presence of such LGL component in murine kidney. Large numbers of LGLs in the kidney would probably have been noted on electron microscopy because of their distinctive granules, although the presence of granules could be dependent on the state of differentiation. Several other considerations argue that these cells are not LGLs. First, in those cases in which a truncated TCR α mRNA of similar size to the one reported in this paper has been documented in cell lines with NK activity, this transcript has always been associated with CD3 expression (40). The finding that only NK cell lines which express CD3 molecules have germline TCR α mRNAs while those that are $CD3^-$ do not express the TCR α gene (40) implies that these cell lines are, in fact, T cell lines with NK activity. However, some of these lines could be of NK origin because it has recently been shown that some NK cells express CD3 ζ as part of the CD16 surface molecule (17, 201). Second, most studies have only found truncated transcripts for β and δ genes, not for TCR α genes (202-205). Nevertheless, the possibility that the truncated TCR α mRNA positive cells are functionally related to the NK or LGL lineage cannot be excluded at present.

Based on the arguments presented above, the most likely origin for the truncated TCR α mRNA is a resident renal cell population. Furthermore, cell fractionation and in situ hybridization experiments suggest that the truncated TCR α mRNA is expressed by a subset of resident renal interstitial cells which are more abundant in the medulla.

The renal interstitium is a dynamic component of the kidney, with resident cells and a complex extracellular matrix embracing fibrillar structures and ground substance which includes proteoglycans, glycoproteins, and interstitial fluid (206). Despite the importance of the interstitium as a critical regulatory element of renal function in health and disease (207), the characterization of renal interstitial cells is still incomplete. Most of our knowledge about resident cell populations in the renal interstitium comes from immunohistochemistry and electron microscopy studies (206). Since 1973, cells in the renal interstitium are classified in four different types: fibroblast-like cells, lipid-laden cells, pericytes, and macrophage-dendritic cells (208). Certain differences between these four types of cells have been reported according to the location of the cells in cortex or medulla (209, 210).

Apart from the macrophage-dendritic cell population of probable bone marrow origin, the other populations of interstitial cells are the fibroblast-like cells and the lipid-laden cells derive from the metanephrogenic mesenchyme (211). The differentiation of this component into interstitial cells is a default process and is regulated by the tips of the developing ureter. These induce the conversion of the mesenchymal stem cells into epithelial cells. Cells that are not induced by the ureteric tips become interstitial cells. Based on the current evidence, it is highly unlikely that the non-bone marrow derived component of the interstitium is responsible for the expression of the truncated TCR α transcript. In particular, the radiosensitivity of the cells expressing this mRNA is not consistent with a non-bone marrow derived component, as it is thought that this component is radioresistant.

The sensitivity to irradiation and the reconstitution with syngeneic bone marrow transplantation after irradiation are the two primordial features of the bone marrow derived component of the renal interstitium (212). In addition, the surface expression of molecules such as class II MHC molecules or LFA molecules, consistently detected in the renal interstitium, supports a bone marrow origin (213, 214). The pattern of expression of the truncated TCR α mRNA in normal mouse kidney is compatible with these characteristics, implying that the cells expressing the truncated TCR α mRNA are bone marrow derived.

From the data presented in this thesis, the most likely candidate for the cellular origin of the truncated TCR α mRNA is the interstitial dendritic cell. Dendritic cells were first described in spleen on the basis of their branching appearance, high class II MHC expression, and potent antigen-presenting ability (215, 216). The Langerhans cells of the skin are probably closely related (217). Subsequently, dendritic cells have been identified in high numbers in thymus-dependent areas of lymphoid organs, and in most non-lymphoid organs (217, 218). In the kidney, dendritic cells have been consistently defined by their expression of class II MHC molecules using anti-class II immunostaining (212, 219-222). This definition is very limited because previous studies on dendritic cells in other organs (217, 223) as well as in the kidney (222) have shown that dendritic cells may be highly heterogeneous on the basis of surface markers. Such heterogeneity in phenotype may be the result of different stages of maturation, different phases of cell activation, location, or lineage of the interstitial cells (223). However, this heterogeneity may also be secondary to technical biases in the preparation of dendritic cell suspensions. Isolation of dendritic cells from non-lymphoid organs is still a technical challenge because of the difficulties of achieving viable cell preparations with high degree of purity. This difficulty is common to isolation procedures for epidermis Langerhans cells and dendritic cells from heart, lung, liver, and gut (216). This problem has been circumvented by the use of different criteria in the isolation procedure such as low buoyant density or lack of adherence to tissue culture surfaces (216). Therefore, the procedure itself may be selecting particular subsets of interstitial cells with particular functional characteristics.

The large renal interstitial cells expressing the truncated TCR α transcript have some similarities to dendritic cells such as their low buoyant density, and their radiosensitivity (212, 217, 224). However, the lack of a pure renal interstitial cell suspension precludes at the present time a more detailed analysis of other similarities between the cells expressing the truncated TCR α mRNA and the interstitial dendritic cells. In the kidney, the presence of different types of epithelial cells and their higher number in comparison to interstitial cells makes isolation of interstitial cells even more difficult than in other organs (see above) and is responsible for the lack of isolation procedures for renal interstitial dendritic cells. The isolation procedure developed in this thesis has not achieved a high degree of purity for the fraction in which the truncated TCR α mRNA is detected. However, the phenotypic pattern detected in the fraction in which the truncated TCR α transcript is detected is also compatible with a dendritic cell lineage as macrophage-dendritic cells express CD45, Mac-1, and class II MHC molecules (216, 217, 224). Nevertheless, it is surprising that class II MHC expression in the truncated TCR α mRNA-rich fraction of renal interstitial cells was relatively low, whereas renal dendritic cells should be class II positive. Pending the development of a technique for purifying the cells expressing the truncated TCR α mRNA and determining their level of class II expression, our tentative conclusion is that these cells may belong to the dendritic cell type which does not express high levels of class II MHC molecules. In addition, if these cells are dendritic cells, then the finding of the truncated TCR α mRNA in the brain is unexpected because dendritic cells have not been detected in the brain (212).

The expression of a truncated TCR α mRNA may lead to the definition of a novel type of interstitial cell found predominantly in the kidney. Compared to lymphoid and blood cell populations, the marrow-derived interstitial cells in the kidney and other epithelial organs remain poorly characterized, due mainly to a lack of definitive isolation and culture procedures. Its selectivity for kidney and brain could reflect selective localization, or selective differentiation due to factors in the renal environment. As these cells are bone marrow derived, then it is likely that the expression of the truncated TCR α mRNA in these cells is dependent upon interaction with the renal microenvironment.

An intriguing aspect of the finding of cells expressing a truncated TCR α mRNA in the kidney and brain is precisely their location. The data presented in this thesis does not provide any hint to address this point. However, it should be taken into consideration that the kidney is a major lymphoid organ in fishes and amphibians, and, in amphibians, the kidney seems to be the source of T and B lymphoid precursors, prior to the liver (225). Although the role of the kidney in lymphopoiesis disappears in the phylogenetic scale, it is possible that such function is still retained by birds (225). Therefore it is possible that the expression of the truncated TCR α mRNA in mouse kidney is a phylogenetic residue, indicative of a restricted cell population of primitive function. This hypothesis would be consistent with our finding that, despite the predominantly T cell-restricted expression of TCR genes, the cells expressing the truncated TCR α mRNA in the kidney are not thymus-dependent. Whether these cells conserve their original function in the mouse kidney or have developed an entirely new role remains unknown.

The hypothesis that the cells expressing the truncated TCR α mRNA represent a phylogenetic residue from primitive lymphopoiesis in the kidney is not satisfactory to explain the expression of the truncated TCR α transcript in the brain. However, some 'lymphoid' surface molecules such as Ly-6 and Thy-1 are expressed in the kidney and in the brain of mice and rats respectively (226). The relevance of this phenomenon is at present unknown.

While the significance of the cell population expressing the truncated TCR α mRNA is unknown, several possibilities must be considered. If these cells are a peculiar type of dendritic cells, then a role in Ag processing and presentation seems possible (218). However, if by virtue of the expression of the TCR α gene, they represent immature stages of lymphocyte development, then it is possible that, under certain circumstances, they may further differentiate (227), with the resulting rearrangement of TCR genes and production of certain cytokines, as is known for dendritic epidermal cells (228).

Based on the pattern of lymphoid-specific expression of TCR genes, the truncated TCR α mRNA may indicate that a population of renal cells has been committed to the early stages of T cell ontogeny, as it has previously been suggested for the truncated TCR γ transcript found in skin dendritic cells of nude mice (229). This explanation does not contradict the central role of the thymus in determining the T cell-specific pattern of expression of TCR genes. However, rearrangement and expression of TCR genes has been documented in in vitro athymic models of differentiation (230), implying that the programmed sequence of TCR gene rearrangement and expression is primarily determined in the stem cell stage, while the thymic environment is critical in determining the progression of T cell differentiation and acquisition of function (231). Some additional evidence supports the existence of an extra-thymic pathway of T cell development: for example, the finding of increasing numbers of T cells in old mice carrying the nude mutation (232). However, we have no indication that the renal cells expressing the truncated TCR α mRNA are in any sense a precursor of T cells.

POSSIBLE FUNCTIONS OF THE TRUNCATED TCR α mRNA AND/OR PRODUCT

The presence of two different mRNA species for TCR α in thymocytes, T cell lines, and PBL has been known since the cloning of the TCR α gene (183, 233, 234). The size of the resulting two bands are 1.8 and 1.3 kb. In PBL, the amount of complete TCR α mRNA is always much higher than the amount of truncated TCR α mRNA (66).

The initial interpretation of the finding of a shorter TCR α mRNA was that the TCR α locus would have D gene segments and that, by analogy with the two different transcripts observed for TCR β (14), the shorter transcript could be the result of a DJ rearrangement. However, no D region has so far been demonstrated for the TCR α locus (3).

Cloning of the shorter TCR α transcripts allowed for a better definition of these transcripts. Analysis of 19 cDNA clones from thymocyte cDNA library hybridizing with a TCR α probe showed that four of them did not contain Vα sequences. These clones were probably representative of the truncated TCR α mRNA. Sequence analysis of the shorter TCR α mRNAs showed that they were heterogenous in sequence. Some of the truncated TCR α transcripts were germline J-C transcripts (40), probably resulting from a promoter located 5' to the particular Jα gene. Others contained TCR α C region with 5' sequences that were not similar to each other or to intronic sequences flanking the C α region (234), and could be the result of aberrant splicing events. Whether the heterogeneity of sequences observed for the truncated TCR α mRNA is specific to the thymus or is a more general feature of this transcript in other stages of T cell differentiation is not known. However, Ig V_H germline transcripts are highly homologous (235), although the transcription starting site is heterogeneous (95, 97, 146, 236, 237).

From the data presented in this thesis, we do not know whether the truncated TCR α mRNA detected in murine kidney is a germline transcript containing only JC regions or is the result of unsuccessful VJ rearrangements. However, several findings imply that this transcript is, in fact, a germline transcript. First, the size of the transcript is identical to the truncated transcript found in thymocytes. Second, the presence of the transcript is not affected by the scid mutation. Although the scid mutation does not affect the initiation of V(D)J recombination, only $D\delta$-$J\delta$ rearrangement attempts are seen in lymphocytes, and the TCR α, TCR β, and TCR γ loci remain in germline configuration (238, 239). A more definitive proof of the germline nature of the transcript may come from the cloning of the transcript itself and from testing its presence in RAG-1 or RAG-2 knock-out mice (79, 80).

The function of germline transcripts of TCR genes in T lymphocytes has not been investigated in detail. However, it is known that a low level of transcription from unrearranged V genes and from J–C regions of Ig (87) and TCR (88) occurs in B and T cells, respectively, prior to the rearrangement process and closely correlates in time with future rearrangements. Specifically, in B cell differentiation, the appearance of germline transcripts precedes the occurrence of gene rearrangement and Ig H chain class switching (91). Therefore, the association of germline transcripts with gene rearrangement has lead to different hypothesis of the role of these transcripts in gene recombination. Five potential functions of germline TCR transcripts will be discussed here: a) the germline transcripts may not have any function; b) the germline transcripts could have a positive regulatory role in TCR gene rearrangement; c) the germline transcripts may code for proteins which are involved in control of TCR gene rearrangement in a positive way; d) the germline TCR transcripts or the resulting proteins may play a role in preventing or negatively controlling TCR gene rearrangement; and e) the germline TCR transcripts may have other functions unrelated to gene rearrangement. Except for the first possibility, the other three are not mutually exclusive and may apply to different germline transcripts.

The germline TCR transcripts may have no function. They may be the result of open conformation of the chromatin to make it accessible to the transcriptional machinery and the recombinase activity required for gene rearrangement or for any transcriptional activity in nearby regions (72, 99). Along the lines of these model, the truncated TCR α mRNA may be the result of 'junk' transcription initiated because of the opening of the chromatin in the region of chromosome 14, where the TCR α/δ locus is located, and required for transcription of relevant genes for kidney function. However, the consistent association of germline transcripts with recombination events in other cell types, the consistent expression of the truncated

TCR α mRNA in different mouse strains, and the considerable processing of the transcript support the idea that the truncated TCR α transcript or its product may play a role in gene rearrangement.

The germline transcripts may have a positive function in gene rearrangement. However, the mechanism by which these transcripts might regulate the recombination process and class switching is not clear. Studies on germ line transcripts for the Ig α H chain gene in a B cell lymphoma showed that the transcription starting site is relatively conserved, although not homogenous, suggesting that the transcript itself or its product may promote class switching (97). The transcript itself may result from a restricted opening of the chromatin and its expression may be necessary for a more spread opening of the chromatin and the induction of rearrangement by formation of intra-molecular triple strands with the switch or RSS regions of DNA (240). Therefore, the germline transcript would direct the recombinase activity through the formation of a particular structure on the DNA. However, some experimental data does not support this conclusion for the truncated TCR α mRNA and some Ig germline transcripts. First, no phenomenon comparable to Ig class switching has been reported for TCR genes yet. Second, it is relatively surprising that the truncated TCR α mRNA or other Ig germline transcripts (235) have to go through full processing and transport to the cytoplasm if their function is to promote gene rearrangement through the formation of particular molecular structures with the DNA. Third, recent experiments show that binding of an antisense phosphorothioate oligonucleotide to the I exon of the

Ig γ2b does not inhibit the expression of germline transcripts but rather increases the expression of these transcripts and inhibits Ig secretion (241). Therefore, the truncated TCR α mRNA in mouse kidney may represent a failed attempt to rearrange the TCR α gene in the cells expressing this transcript rather than promoting TCR α gene rearrangement.

A third possibility is that the transcript could be translated into a protein which would have a positive function in gene rearrangement. Evidence in favour of this model comes from the demonstration that germline transcripts from Ig genes, and more recently from TCR α V genes, have open reading frames that code for small peptides (97, 98). For the TCR α V germline transcripts, its has been preliminarily shown that, in the thymus, transcripts for the Vα3 and Vα8 subfamily members have conservation of coding regions and 3' flanking regions. Examination of the predicted aa sequences of the open reading frame of one of the Vα3 germline transcripts revealed the presence of striking homology with metal binding domains in proteins. In contrast, the open reading frames of some Vα8 germline transcripts terminated with a motif involved in prenylation of the protein product (which involves the addition of lipid residues in post-translational modification) (98). None of these motifs has a known role in gene rearrangement. Therefore, these proteins may have currently unknown roles in gene rearrangement or some additional functions unrelated to the recombination process.

One distinctive feature of the truncated TCR α mRNA expressed in normal mouse kidney is that its expression is not associated with future rearrangements of the TCR α gene, at least under physiological circumstances, or with detectable expression of other TCR genes. This is in contrast to the presence of germline transcripts in T and B cell precursors. If the previous models proposing a role of the germline transcripts in the progression of gene rearrangement are correct, then it is not clear why TCR α gene rearrangement is stopped in the renal interstitial cells expressing the truncated TCR α mRNA. Because transcription of rearranged TCR genes requires full enhancer activity, the arrest in the pre-rearrangement stage of TCR gene transcription may be due to the lack of factor/s necessary for full enhancer activity and subsequent successful rearrangement (101, 107). The absence of similar transcripts for the TCR β gene could be explained by different mechanisms of control for the TCR α and β genes, as suggested in previous reports (139-141).

Alternatively, the function of the truncated TCR α mRNA in the kidney may be to control TCR α gene rearrangement in a negative manner rather than in a positive manner. A negative role of germline transcripts on gene rearrangement and cell maturation has been reported recently (199). It relates to the role of Dμ protein in B cell differentiation. Mature B cells have a preferential usage of reading frame one in the D-J junction of Ig μ H chain. The use of this particular reading frame is not predominant during early stages, in particular in pre-B cells in which truncated DJ-C transcripts for the Ig μ H chain are present. Analysis of clones expressing these transcripts and using reading frame three shows, however, that this reading frame has multiple stop codons in its sequence, leading to termination of translation. However, when clones using reading frame two were analyzed, it was shown that this reading frame is an open reading frame which codes for a protein, known as Dμ protein. From these findings, Gu et al suggested that the expression of the Dμ protein had a role in controlling Ig H chain gene rearrangement. In particular, the expression of the Dμ protein in pre-B cells would be associated with arresting B cell differentiation by stopping full rearrangement of the Ig H chain locus. To test the hypothesis that the function of the Dμ protein requires its surface expression, they produced mice transgenic for the Dμ protein with a disruption of the membrane exon of the Cμ gene. In these mice, the preferential usage of D-J reading frame one in mature B cells was not observed, indicating that the membrane-bound Dμ protein plays a role in the regulation of B cell development by stopping the maturation of pre-B cells expressing Dμ protein.

The current data on the truncated TCR α mRNA expressed in normal mouse kidney is compatible with the model proposed by Gu et al. The truncated TCR α mRNA is never associated with expression of complete TCR α mRNA or with expression of TCR β mRNA. The transcript is translated into a protein. On the basis of partial sequence of the truncated TCR α mRNA, the resulting truncated TCR α protein contains an intact transmembrane domain. Finally, preliminary experiments show that in vivo administration of cycloheximide, attempting to block translation of the truncated TCR α mRNA, correlates with expression of complete TCR α mRNA. Although the effects of in vivo administration of cycloheximide may be multiple, acting at different levels of regulation of gene expression, no effect of cycloheximide on TCR α gene transcription has so far been demonstrated. In vitro, cycloheximide induces an increase in the steady state levels of functional TCR α mRNA but current evidence indicates that this increase is secondary to nuclear or cytoplasmic post-transcriptional events (140).

The truncated TCR α mRNA in the kidney may have other functions not related to control of TCR α gene rearrangement, as already mentioned above (98). One possible function could be the use of the truncated TCR α mRNA as a substrate for trans-splicing with functional TCR α transcripts like it has been already reported for Ig germline transcripts (242, 243). This possibility is unlikely in the case of the truncated TCR α mRNA in normal mouse kidney as no evidence for expression of complete TCR transcripts in this organ has been obtained. Another possible function for the truncated TCR α mRNA and/or protein could be local immunoregulation. Recently, some evidence has been reported on the potential role of the TCR α chain in regulation of the immune response, specifically, as an Ag-binding factor (244-250). The Ag-specific binding activity correlates with expression of a complete TCR α chain, but not with expression of TCR β chain, as blocking translation of TCR α mRNA with an specific antisense oligonucleotide abolishes Ag binding (245), and reconstitution of TCR$^-$ variants of T cell hybridomas with TCR α cDNA but not with TCR β cDNA restores Ag-binding activity (250). A soluble form of TCR α chain is also active as an Ag-binding factor (249). However, it is not clear how the TCR α chain is released from the cell as there is no evidence of alternative splicing for the TCR α mRNA and the constructs used in reconstitution experiments contained intact transmembrane domains (250). The possibility that the truncated TCR α mRNA expressed in the kidney has a function related to suppressor immunoregulatory activity is unlikely. The immunoregulatory activity reported for the TCR α chain is related to expression of a complete TCR α chain and on its expression of T cells, two characteristics that are not met by the truncated TCR α mRNA.

A HYPOTHESIS FOR THE FUNCTION OF THE TRUNCATED TCR α mRNA EXPRESSED IN MOUSE KIDNEY

On the basis of the data presented thus far, our hypothesis on the significance and function of the truncated TCR α mRNA in normal mouse kidney is that this transcript is indicative of the presence of a pre-lymphoid cell population in the kidney, probably representing a residue from phylogeny. The truncated TCR α mRNA leads to the expression of a truncated TCR α protein on the surface of these particular renal cells. The expression of this protein is responsible for preventing rearrangement of the TCR α gene in these cells. How this may be accomplished is not clear, considering that this hypothetical function is targeted at the nucleus and the truncated TCR α protein is likely a transmembrane protein. It may be that the engagement of a hypothetical ligand to this molecule is required for negative signalling from the truncated TCR α protein to prevent TCR α gene rearrangement. Alternatively, the presence of the hypothetical ligand and its engagement with the truncated TCR α protein under certain circumstances may lead to TCR α gene rearrangement.

FUTURE PERSPECTIVES

The results presented in this thesis open multiple avenues to future research because several aspects of the expression of a truncated TCR α mRNA in normal mouse kidney need still to be explored.

In my opinion, the first major step should be the cloning of the whole truncated TCR α mRNA and the verification by RNase protection assay that the clone is representative of the majority of TCR α transcripts in mouse kidney. The information that this would provide is essential to clarify the nature of the transcript. Two main possible results could be expected. It may be that the transcript is a germline transcript, starting in the intron 5' to the beginning of the TCR α C gene. On the other hand, it could be that the truncated TCR α mRNA is the result of abnormal rearrangements involving one or more J genes and/or abnormal splicing within the J-C intron.

If the truncated TCR α mRNA is a germline transcript, then it would be very interesting to define the regulation of its transcription and, in particular, the presence of cryptic promoters in the vicinity of the transcription starting site. The nature of cryptic promoters remains a totally unexplored area in regulation of gene expression.

If the truncated TCR α mRNA is an abnormally spliced transcript, effort could be concentrated in the mapping and study of the role of secondary splicing signals.

Once the truncated TCR α mRNA has been fully cloned, experiments can be designed to explore the function of the transcript, including transfection studies as well as homologous recombination studies, either in mature T cells or in T cell precursors.

The second major avenue in the future development of this project should concentrate in the isolation, purification, and characterization of the cells expressing the truncated TCR α mRNA, and ideally in the establishment of long term culture conditions. The availability of cell cultures would facilitate future studies regarding the analysis of the function of the transcript as well as the differentiation potential. However, the possibility of an isolation procedure for the cells expressing the transcript would also be of great interest for studies of the differentiation potential of these cells in vivo, with the hypothesis that the cells expressing the truncated TCR α mRNA may be able to undergo further differentiation along the T cell lineage, establishing a model of extrathymic development of T cells. A parallel approach could be the use of retrovirus-infected haematopoietic stem cells with the truncated TCR α mRNA inserted in the retroviral vector. This approach has been shown to be useful to achieve long-term expression of a TCR gene in a lymphoid-specific manner (251). In this particular model, it would be of interest to test the expression of TCR genes in the kidney, and, if this is the case, the potential interactions of the TCR chains being produced in the renal interstitium.

CHAPTER FIVE:

CONCLUSIONS

The results presented in this thesis demonstrate that:

1.- Normal mouse kidney expresses a truncated TCR α mRNA of 1.3-1.4 kb which is not associated with expression of complete TCR α mRNA (1.7 kb) or to expression of other TCR genes (β or δ).

2.- The truncated TCR α mRNA contains a correctly spliced Cα region as indicated by TCR α C region oligonucleotide probing, PCR amplification of the Cα region from kidney total RNA, sequencing of and hybridization with the amplified products, and RNase protection assay.

3.- The truncated TCR α mRNA is expressed by large renal interstitial cells that are more abundant in the renal medulla and are not mature T cells (in that they are large, low buoyant density cells which isolate in a fraction with no detectable expression of complete TCR α, TCR ß, CD3 and Thy-1) or NK cells (because they do not express the NK cell marker NK-1.1).

4.- The expression of the truncated TCR α mRNA is radiosensitive in a dose dependent manner, and can be restored after reconstitution with bone marrow cells indicating that the cells expressing this transcript are bone marrow dependent. However, the transcript is not expressed in the adult bone marrow, implying that the expression of the truncated TCR α mRNA is dependent upon interaction with the renal microenvironment.

5.- The expression of the truncated TCR α mRNA is not thymus-dependent and does not require a functional recombinase activity as the levels of the transcript in the kidney are not affected by the nude or the scid mutations.

6.- The truncated TCR α mRNA is normally processed, being correctly spliced at the TCR α C region, polyadenylated, and exported to the cytoplasm.

7.- Immunoaffinity precipitation suggests that the truncated TCR α mRNA is translated into a ~30 kd protein that, on the basis of conservation of the transmembrane domain of the Cα gene, may be a transmembrane protein.

8.- In vivo administration of cycloheximide is associated with the appearance of a 1.7 kd band which hybridizes with a TCR α probe in the kidney and slight decrease of the levels of truncated TCR α mRNA, without any evidence of renal T cell infiltration as indicated with immunohistochemistry and northern blotting. The size of the 1.7 kd band is compatible with the size of a complete TCR α transcript. This result suggests that blocking of translation of the truncated TCR α mRNA correlates with rearrangement of the TCR α gene.

BIBLIOGRAPHY

1. **Allison, J. P., and L. L. Lanier**. 1987. Structure, Function, and Serology of the T-Cell Antigen Receptor Complex. *Annu. Rev. Immunol. 5:503.*

2. **Clevers, H., B. Alarcon, T. Wileman, and C. Terhorst**. 1988. The T Cell Receptor/CD3 Complex: A Dynamic Protein Ensemble. *Annu. Rev. Immunol. 6:629.*

3. **Davis, M. M., and P. J. Bjorkman**. 1988. T-Cell Antigen Receptor Genes and T-Cell Recognition. *Nature 334:395.*

4. **Wilson, R. K., E. Lai, P. Concannon, R. K. Barth, and L. E. Hood**. 1988. Structure, Organization and Polymorphism of Murine and Human T-Cell Receptor α and β Chain Gene Families. *Immunol. Rev. 101:149.*

5. **Weiss, A**. 1990. Structure and Function of the T Cell Antigen Receptor. *J. Clin. Invest. 86:1015.*

6. **Reth, M., J. Hombach, J. Wienands, K. S. Campbell, N. Chien, L. B. Justement, and J. C. Cambier**. 1991. The B-Cell Antigen Receptor Complex. *Immunol. Today 12:196.*

7. **Rolink, A., and F. Melchers**. 1991. Molecular and Cellular Origins of B Lymphocyte Diversity. *Cell 66:1081.*

8. **Kadesch, T**. 1992. Helix-Loop-Helix Proteins in the Regulation of Immunoglobulin Gene Transcription. *Immunol. Today 13:31.*

9. **Hedrick, S. M., E. A. Nielsen, J. Kavaler, D. I. Cohen, and M. M. Davis**. 1984. Sequence Relationships Between Putative T-Cell Receptor Polypeptides and Immunoglobulins. *Nature 308:153.*

10. **Jensenius, J. C., and A. F. Williams**. 1982. The T Lymphocyte Antigen

Receptor - Paradigm Lost. *Nature 300:583.*

11. **Allison, J. P., B. W. McIntyre, and D. Bloch**. 1982. Tumor-Specific Antigen of Murine T Lymphoma Defined with Monoclonal Antibody. *J. Immunol. 129:2293.*

12. **Haskins, K., R. Kubo, J. White, M. Pigeon, J. Kappler, and P. Marrack**. 1983. The Major Histocompatibility Complex-Restricted Antigen Receptor on T Cells. I. Isolation with a Monoclonal Antibody. *J. Exp. Med. 157:1149.*

13. **Meuer, S., K. Fitzgerald, R. Hussey, J. Hodgdon, S. F. Schlossman, and E. L. Reinherz**. 1983. Clonotypic Structures Involved in Antigen-Specific Human T Cell Function. Relationship to the T3 Molecular Complex. *J. Exp. Med. 157:705.*

14. **Yanagi, Y., Y. Yoshikai, K. Leggett, S. P. Clark, I. Aleksander, and T. W. Mak**. 1984. A Human T Cell-Specific cDNA Clone Encodes a Protein Having Extensive Homology to Immunoglobulin Chains. *Nature 308:145.*

15. **Hedrick, S. M., D. I. Cohen, E. A. Nielsen, and M. M. Davis**. 1984. Isolation of cDNA Clones Encoding T Cell-Specific Membrane-Associated Proteins. *Nature 308:149.*

16. **Strominger, J. L**. 1989. Developmental Biology of T Cell Receptors. *Science 244:943.*

17. **Clevers, H. C., and M. J. Owen**. 1991. Towards a Molecular Understanding of T-Cell Differentiation. *Immunol. Today 12:86.*

18. **Marrack, P., and J. Kappler**. 1987. The T Cell Receptor. *Science 238:1073.*

19. **Raulet, D. H.** 1989. The Structure, Function, and Molecular Genetics of the γ/δ T Cell Receptor. *Annu. Rev. Immunol. 7:175.*

20. **Chothia, C., D. R. Boswell, and A. M. Lesk**. 1988. The Outline Structure of the T-Cell $\alpha\beta$ Receptor. *EMBO J. 7:3745.*

21. **Jorgensen, J. L., U. Esser, B. Fazekas de St. Groth, P. A. Reay, and M. M. Davis**. 1992. Mapping T-Cell Receptor-Peptide Contacts by Variant Peptide Immunization of Single-Chain Transgenics. *Nature 355:224.*

22. **Saito, T., F. Hochstenbach, S. Marusic-Galesic, A. Kruisbeek, M. Brenner, and R. N. Germain**. 1988. Surface Expression of Only $\gamma\delta$ and/or $\alpha\beta$ T Cell Receptor Heterodimers by Cells with Four (α, β, γ, δ) Functional Receptor Chains. *J. Exp. Med. 168:1003.*

23. **Brenner, M. B., J. McLean, H. Scheft, J. Riberdy, S.-L. Ang, J. G. Seidman, P. Devlin, and M. S. Krangel**. 1987. Two Forms of the T-Cell Receptor γ Protein Found on Peripheral Blood Cytotoxic T Lymphocytes. *Nature 325:689.*

24. **Borst, J., J. J. M. van Dongen, R. L. H. Bolhuis, P. J. Peters, D. A. Hafler, E. de Vries, and R. J. van de Griend**. 1988. Distinct Molecular Forms of Human T Cell Receptor γ/δ Detected on Viable T Cells by a Monoclonal Antibody. *J. Exp. Med. 167:1625.*

25. **Hochstenbach, F., C. Parker, J. McLean, V. Gieselmann, H. Band, I. Bank, L. Chess, H. Spits, J. L. Strominger, J. G. Seidman, and M. B. Brenner**. 1988. Characterization of a Third Form of the Human T Cell Receptor γ/δ. *J. Exp. Med. 168:761.*

26. **Triebel, F., and T. Hercend**. 1989. Subpopulations of Human Peripheral T Gamma Delta Lymphocytes. *Immunol. Today 10:186.*

27. **Brenner, M. B., J. McLean, D. P. Dialynas, J. L. Strominger, J. A. Smith, F. L. Owen, J. G. Seidman, S. Ip, F. Rosen, and M. S. Krangel**. 1986. Identification of a Putative Second T-Cell Receptor. *Nature 322:145.*

28. **Borst, J., R. J. van de Griend, J. W. van Oostveen, S.-L. Ang, C. J. Melief, J. G. Seidman, and R. L. H. Bolhuis**. 1987. A T-Cell Receptor γ/CD3 Complex Found on Cloned Functional Lymphocytes. *Nature 325:683.*

29. **Hochstenbach, F., and M. B. Brenner**. 1989. T-Cell Receptor δ-Chain Can Substitute for α to Form a $\beta\delta$ Heterodimer. *Nature 340:562.*

30. **von Boehmer, H**. 1990. Developmental Biology of T Cells in T Cell-Receptor Transgenic Mice. *Annu. Rev. Immunol. 8:531.*

31. **Kishi, H., P. Borgulya, B. Scott, K. Karjalainen, A. Traunecker, J. Kaufman, and H. von Boehmer**. 1991. Surface Expression of the β T Cell Receptor (TCR) Chain in the Absence of Other TCR or CD3 Proteins on Immature T Cells. *EMBO J. 10:93.*

32. **Allison, J. P., and W. L. Havran**. 1991. The Immunobiology of T Cells with Invariant $\gamma\delta$ Antigen Receptors. *Annu. Rev. Immunol. 9:679.*

33. **Stingl, G., F. Koning, H. Yamada, W. M. Yokohama, E. Tschachler, J. A. Bluestone, G. Steiner, L. E. Samelson, A. M. Lew, and J. E. Coligan**. 1987. Thy-1^+ Dendritic Epidermal Cells Express T3 Antigen and the T-Cell Receptor Gamma Chain. *Proc. Natl. Acad. Sci. USA. 84:4586.*

34. **Stingl, G., K. C. Gunter, E. Tschachler, H. Yamada, R. I. Lechler, W. M. Yokohama, G. Steiner, R. N. Germain, and E. M. Shevach**. 1987. Thy-1^+

Dendritic Epidermal Cells Belong to the T-Cell Lineage. *Proc. Natl. Acad. Sci. USA. 84:2430.*

35. **Kuziel, W. A., A. Takashima, M. Bonyhadi, P. R. Bergstresser, J. P. Allison, R. E. Tigelaar, and P. W. Tucker**. 1987. Regulation of T-Cell Receptor γ-Chain RNA Expression in Murine Thy-1^+ Dendritic Epidermal Cells. *Nature 328:263.*

36. **Goodman, T., and L. Lefrancois**. 1988. Expression of the γ/δ T-Cell Receptor on Intestinal CD8^+ Intraepithelial Lymphocytes. *Nature 333:855.*

37. **Lin, A. Y., B. Devaux, A. Green, C. Sagerström, J. F. Elliott, and M. M. Davis**. 1990. Expression of T Cell Antigen Receptor Heterodimers in a Lipid-Linked Form. *Science 249:677.*

38. **Wegener, A. K., F. Letourneur, A. Hoeveler, T. Brocker, F. Luton, and B. Malissen**. 1992. The T Cell Receptor/CD3 Complex is Composed of at Least Two Autonomous Transduction Modules. *Cell 68:83.*

39. **Kappes, D. J., and S. Tonegawa**. 1991. Surface Expression of Alternative Forms of the TCR/CD3 Complex. *Proc. Natl. Acad. Sci. USA. 88:10619.*

40. **Kronenberg, M., G. Siu, L. E. Hood, and N. Shastri**. 1986. The Molecular Genetics of the T-Cell Antigen Receptor and T-Cell Antigen Recognition. *Annu. Rev. Immunol. 4:529.*

41. **Toyonaga, B., and T. W. Mak**. 1987. Genes of the T-Cell Antigen Receptor in Normal and Malignant T Cells. *Annu. Rev. Immunol. 5:585.*

42. **Satyanarayana, K., S. Hata, P. Devlin, M. G. Roncarolo, J. E. De Vries,**

H. Spits, J. L. Strominger, and M. S. Krangel. 1988. Genomic Organization of the Human T-Cell Antigen Receptor α/δ Locus. *Proc. Natl. Acad. Sci. USA. 85:8166.*

43. **Griesser, H., E. Champagne, D. Tkachuk, Y. Takihara, M. Lalande, E. Baillie, M. Minden, and T. W. Mak**. 1988. The Human T Cell Receptor α-δ Locus: A Physical Map of the Variable, Joining and Constant Region Genes. *Eur. J. Immunol. 18:641.*

44. **Chien, Y., M. Iwashima, K. B. Kaplan, J. F. Elliott, and M. M. Davis**. 1987. A New T-Cell Receptor Gene Located Within the Alpha Locus and Expressed Early in T-Cell Differentiation. *Nature 327:677.*

45. **Behlke, M. A., H. S. Chou, K. Huppi, and D. Y. Loh**. 1986. Murine T-Cell Receptor Mutants with Deletions of β-Chain Variable Region Genes. *Proc. Natl. Acad. Sci. USA. 83:767.*

46. **Chou, H. S., S. J. Anderson, M. C. Louie, S. A. Godambe, M. R. Pozzi, M. A. Behlke, K. Huppi, and D. Y. Loh**. 1987. Tandem Linkage and Unusual RNA Splicing of the T-Cell Receptor β-Chain Variable-Region Genes. *Proc. Natl. Acad. Sci. USA. 84:1992.*

47. **Lamoyi, E., A. L. Angiolillo, and R. G. Mage**. 1986. An Evolutionary Conserved Rabbit T-Cell Receptor β-Chain Variable Region. *Immunogenetics 23:266.*

48. **Williams, C. B., and G. A. Gutman**. 1989. T Cell Receptor β-Chain Genes in the Rat. Availability and Pattern of Utilization of V Gene Segments Differs from

that in the Mouse. *J. Immunol. 142:1027.*

49. **Wilson, R. K., E. Lai, L. D. Kim, and L. Hood**. 1990. Sequence and Expression of a Novel Human T-Cell Receptor β-Chain Variable Gene Segment Subfamily. *Immunogenetics 32:406.*

50. **Robinson, M. A**. 1991. The Human T Cell Receptor β-Chain Gene Complex Contains at Least 57 Variable Gene Segments. Identification of Six Vβ Genes in Four New Gene Families. *J. Immunol. 146:4392.*

51. **Plaza, A., D. H. Kono, and A. N. Theofilopoulos**. 1991. New Human Vβ Genes and Polymorphic Variants. *J. Immunol. 147:4360.*

52. **Malissen, M., C. McCoy, D. Blanc, J. Trucy, C. Devaux, A. Schmitt-Verhulst, F. Fitch, L. Hood, and B. Malissen**. 1986. Direct Evidence for Chromosomal Inversion during T-Cell Receptor β-Gene Rearrangements. *Nature 319:28.*

53. **Iwashima, M., A. Green, M. M. Davis, and Y. Chien**. 1988. Variable Region (Vδ) Gene Segment Most Frequently Utilized in Adult Thymocytes is 3' of the Constant (Cδ) Region. *Proc. Natl. Acad. Sci. USA. 85:8161.*

54. **Korman, A. J., J. Maruyama, and D. H. Raulet**. 1989. Rearrangement by Inversion of a T-Cell Receptor δ Variable Region Gene Located 3' of the δ Constant Region Gene. *Proc. Natl. Acad. Sci. USA. 86:267.*

55. **Elliott, J. F., E. P. Rock, P. A. Patten, M. M. Davis, and Y. Chien**. 1988. The adult T-Cell Receptor δ-Chain is Diverse and Distinct from that of Fetal Thymocytes. *Nature 331:627.*

56. **Chien, Y., M. Iwashima, D. A. Wettstein, K. B. Kapplan, J. F. Elliott, W. Born, and M. M. Davis**. 1987. T Cell Receptor δ Gene Rearrangements in Early Thymocytes. *Nature 330:722.*

57. **Williams, A. F., and A. N. Barclay**. 1988. The Immunoglobulin Superfamily - Domains for Cell Surface Recognition. *Annu. Rev. Immunol. 6:381.*

58. **Dent, A. L., P. J. Fink, and S. M. Hedrick**. 1989. Characterization of an Alternative Exon of the Murine T Cell Receptor β-Chain. Pattern of Expression and Evolutionary Conservation. *J. Immunol. 143:322.*

59. **Williams, C. B., E. P. Blankenhorn, K. E. Byrd, G. Levinson, and G. A. Gutman**. 1991. Organization and Nucleotide Sequence of the Rat T Cell Receptor β-Chain Complex. *J. Immunol. 146:4406.*

60. **Tonegawa, S**. 1983. Somatic Generation of Antibody Diversity. *Nature 302:575.*

61. **Lewis, S., and M. Gellert**. 1989. The Mechanism of Antigen Receptor Gene Assembly. *Cell 59:585.*

62. **Lafaille, J. J., A. DeCloux, M. Bonneville, Y. Takagaki, and S. Tonegawa**. 1989. Juntional Sequences of T Cell Receptor γδ Genes: Implications for γδ T Cell Lineages and for a Novel Intermediate of V-(D)-J Joining. *Cell 59:859.*

63. **von Boehmer, H**. 1988. The Developmental Biology of T Lymphocytes. *Annu. Rev. Immunol. 6:309.*

64. **Winoto, A**. 1991. Regulation of the Early Stages of T-Cell Development. *Curr. Opin. Immunol. 3:199.*

65. **Raulet, D. H., R. D. Garman, H. Saito, and S. Tonegawa**. 1985. Developmental Regulation of T-Cell Receptor Gene Expression. *Nature 314:103.*

66. **Snodgrass, H. R., Z. Dembic, M. Steinmetz, and H. von Boehmer**. 1985. Expression of T-Cell Antigen Receptor Genes during Fetal Development in the Thymus. *Nature 315:232.*

67. **Havran, W. L., and J. P. Allison**. 1988. Developmentally Ordered Appearance of Thymocytes Expressing Different T-Cell Antigen Receptors. *Nature 335:443.*

68. **Krangel, M. S., H. Yssel, C. Brocklehurst, and H. Spits**. 1990. A Distinct Wave of Human T Cell Receptor γ/δ Lymphocytes in the Early Fetal Thymus: Evidence for Controlled Gene Rearrangement and Cytokine Production. *J. Exp. Med. 172:847.*

69. **McVay, L. D., S. R. Carding, K. Bottomly, and A. C. Hayday**. 1991. Regulated Expression and Structure of T Cell Receptor γ/δ Transcripts in Human Thymic Ontogeny. *EMBO J. 10:83.*

70. **Lanier, E. R., R. M. Brown, and E. Kraig**. 1991. Binding of Thymic Factors to the Conserved Decanucleotide Promoter Element of the T-Cell Receptor V_β Gene is Developmentally Regulated and is Absent in SCID Mice. *Proc. Natl. Acad. Sci. USA. 88:8131.*

71. **Barnes, D. E., A. E. Tomkinson, A. R. Lehmann, A. D. B. Webster, and T. Lindahl**. 1992. Mutations in the DNA Ligase I Gene of an Individual with Immunodeficiencies and Cellular Hypersensitivity to DNA-Damaging Agents. *Cell*

69:495.

72. **Yancopoulos, G. D., T. K. Blackwell, H. Suh, L. Hood, and F. W. Alt**. 1986. Introduced T Cell Receptor Variable Region Gene Segments Recombine in Pre-B Cells: Evidence that B and T Cells Use a Common Recombinase. *Cell 44:251.*

73. **Schatz, D. G., and D. Baltimore**. 1988. Stable Expression of Immunoglobulin Gene V(D)J Recombinase Activity by Gene Transfer into 3T3 Fibroblasts. *Cell 53:107.*

74. **Schatz, D. G., M. A. Oettinger, and D. Baltimore**. 1989. The V(D)J Recombination Activating Gene, RAG-1. *Cell 59:1035.*

75. **Oettinger, M. A., D. G. Schatz, C. Gorka, and D. Baltimore**. 1990. RAG-1 and RAG-2, Adjacent Genes that Synergistically Activate V(D)J Recombination. *Science 248:1517.*

76. **Chun, J. J. M., D. G. Schatz, M. A. Oettinger, R. Jaenisch, and D. Baltimore**. 1991. The Recombination Activating Gene-1 (RAG-1) Transcript is Present in the Murine Central Nervous System. *Cell 64:189.*

77. **Carlson, L. M., M. A. Oettinger, D. G. Schatz, E. L. Masteller, E. A. Hurley, W. T. McCormack, D. G. Baltimore, and C. B. Thompson**. 1991. Selective Expression of RAG-2 in Chicken B Cells Undergoing Immunoglogulin Gene Conversion. *Cell 64:201.*

78. **Matsuoka, M., F. Nagawa, K. Okazaki, L. Kingsbury, K. Yoshida, U. Müller, D. T. Larue, J. A. Winer, and H. Sakano**. 1991. Detection of Somatic

DNA Recombination in the Transgenic Mouse Brain. *Science 254:81.*

79. **Mombaerts, P., J. Iacomini, R. S. Johnson, K. Herrup, S. Tonegawa, and V. E. Papaioannou**. 1992. RAG-1-Deficient Mice Have no Mature B and T Lymphocytes. *Cell 68:869.*

80. **Shinkai, Y., G. Rathbun, K.-P. Lam, E. M. Oltz, V. Stewart, M. Mendelsohn, J. Charron, M. Datta, F. Young, A. M. Stall, and F. W. Alt**. 1992. RAG-2-Deficient Mice Lack Mature Lymphocytes Owing to Inability to Initiate V(D)J Rearrangement. *Cell 68:855.*

81. **Roth, D. B., P. B. Nakajima, J. P. Menetski, M. J. Bosma, and M. Gellert**. 1992. V(D)J Recombination in Mouse Thymocytes: Double-Strand Breaks near T Cell Receptor δ Rearrangement Signals. *Cell 69:41.*

82. **Jameson, S. C., P. B. Nakajima, J. L. Brooks, W. Heath, O. Kanagawa, and N. R. J. Gascoigne**. 1991. The T Cell Receptor Vα11 Gene Family. Analysis of Allelic Sequence Polymorphism and Demonstration of J α Region-Dependent Recognition by Allele-Specific Antibodies. *J. Immunol. 147:3185.*

83. **Tycko, B., J. D. Palmer, and J. Sklar**. 1989. T Cell Receptor Gene Trans-Rearrangements: Chimeric γ-δ Genes in Normal Lymphoid Tissues. *Science 245:1242.*

84. **Tycko, B., H. Coyle, and J. Sklar**. 1991. Chimeric γ-δ Signal Joints. Implications for the Mechanism and Regulation of T Cell Receptor Gene Rearrangement. *J. Immunol. 147:705.*

85. **Felsenfeld, G.** 1992. Chromatin as an Essential Part of the Transcriptional Machinery. *Nature 355:219.*

86. **Calame, K., and S. Eaton.** 1988. Transcriptional Controlling Elements in the Immunoglobulin and T Cell Receptor Loci. *Adv. Immunol. 43:235.*

87. **Blackwell, T. K., M. W. Moore, G. D. Yancopoulos, H. Suh, S. Lutzker, E. Selsing, and F. W. Alt.** 1986. Recombination between Immunoglobulin Variable Region Gene Segments is Enhanced by Transcription. *Nature 324:585.*

88. **Ferrier, P., B. Krippl, T. K. Blackwell, A. J. W. Furley, H. Suh, A. Winoto, W. D. Cook, L. Hood, F. Costantini, and F. W. Alt.** 1990. Separate Elements Control DJ and VDJ Rearrangement in a Transgenic Recombination Substrate. *EMBO J. 9:117.*

89. **Fondell, J. D., J. P. Marolleau, D. Primi, and K. B. Marcu.** 1990. On the Mechanism of Non-Allelically Excluded V_α-J_α T Cell Receptor Secondary rearrangements in a Murine T Cell Lymphoma. *J. Immunol. 144:1094.*

90. **Esser, C., and A. Radbruch.** 1990. Immunoglobulin Class Switching: Molecular and Cellular Analysis. *Annu. Rev. Immunol. 8:717.*

91. **Staudt, L. M., and M. J. Lenardo.** 1991. Immunoglobulin Gene Transcription. *Annu. Rev. Immunol. 9:373.*

92. **Schlissel, M. S., and D. Baltimore.** 1989. Activation of Immunoglobulin Kappa Gene Rearrangement Correlates with Induction of Germline Kappa Gene Transcription. *Cell 58:1001.*

93. **Martin, D., R. Huang, T. LeBien, and B. Van Ness.** 1991. Induced

Rearrangement of k Genes in the BLIN-1 Human Pre-B Cell Line Correlates with Germline J-C_k and V_k Transcription. *J. Exp. Med. 173:639.*

94. **Stavnezer, J., G. Radcliffe, Y.-C. Lin, J. Nietupski, L. Berggren, R. Sitia, and E. Severinson**. 1988. Immunoglobulin Heavy-Chain Switching May Be Directed by Prior Induction of Transcripts from Constant-Region Genes. *Proc. Natl. Acad. Sci. USA. 85:7704.*

95. **Kuze, K., A. Shimizu, and T. Honjo**. 1991. Characterization of the Enhancer Region for Germline Transcription of the Gamma 3 Constant Region Gene of Human Immunoglobulin. *Int. Immunol. 3:647.*

96. **Alt, F. W., T. K. Blackwell, R. A. DePinho, M. G. Reth, and G. D. Yancopoulos**. 1986. Regulation of Genome Rearrangement Events during Lymphocyte Differentiation. *Immunol. Rev. 89:5.*

97. **Radcliffe, G., Y.-C. Lin, M. Julius, K. B. Marcu, and J. Stavnezer**. 1990. Structure of Germ Line Immunoglobulin α Heavy-Chain RNA and Its Location on Polysomes. *Mol. Cell. Biol. 10:382.*

98. **Kranz, D. M., P. O. Holman, M. Huang, K. N. Tan, and M. E. Roth**. 1992. Germline Transcripts from Some T Cell Receptor Variable Region Genes May Encode Proteins with Novel Functions. *FASEB J. 6:A1976.*

99. **McDougall, S., C. L. Peterson, and K. Calame**. 1988. A Transcriptional Enhancer 3' of $C_{\beta 2}$ in the T Cell Receptor β Locus. *Science 241:205.*

100. **Ho, I.-C., L.-H. Yang, G. Morle, and J. M. Leiden**. 1989. A T-Cell-Specific Transcriptional Enhancer Element 3' of C_α in the Human T-Cell Receptor α Locus.

Proc. Natl. Acad. Sci. USA. 86:6714.

101. **Winoto, A., and D. Baltimore**. 1989. A Novel, Inducible and T Cell-Specific Enhancer Located at the 3' End of the T Cell Receptor α Locus. *EMBO J. 8:729.*

102. **Ho, I.-C., and J. M. Leiden**. 1990. Regulation of the Human T-Cell Receptor α Gene Enhancer: Multiple Ubiquitous and T-Cell-Specific Nuclear Proteins Interact with Four Hypomethylated Enhancer Elements. *Mol. Cell. Biol. 10:4720.*

103. **Maniatis, T., S. Goodburn, and J. A. Fischer**. 1987. Regulation of Inducible and Tissue-Specific Gene Expression. *Science 236:1237.*

104. **Mitchell, P. J., and R. Tjian**. 1989. Transcriptional Regulation in Mammalian Cells by Sequence-Specific DNA Binding Proteins. *Science 245:371.*

105. **Leiden, J. M**. 1992. Transcriptional Regulation During T-Cell Development: the α TCR Gene as a Molecular Model. *Immunol. Today 13:22.*

106. **Siu, G., E. C. Strauss, E. Lai, and L. E. Hood**. 1986. Analysis of a Human V_β Gene Subfamily. *J. Exp. Med. 164:1600.*

107. **Luria, S., G. Gross, M. Horowitz, and D. Givol**. 1987. Promoter and Enhancer Elements in the Rearranged α Chain Gene of the Human T Cell Receptor. *EMBO J. 6:3307.*

108. **Royer, H. D., and E. L. Reinherz**. 1987. Multiple Nuclear Proteins Bind Upstream Sequences in the Promoter region of a T-Cell Receptor β-Chain Variable Region Gene: Evidence for Tissue Specificity. *Proc. Natl. Acad. Sci. USA. 84:232.*

109. **Lee, N. E., and M. M. Davis**. 1988. T Cell Receptor β-Chain Genes in BW5147 and Other AKR Tumors. Deletion Order of Murine V_β Gene Segments and

Possible 5' Regulatory Regions. *J. Immunol. 140:1665.*

110. **Anderson, S. J., H. S. Chou, and D. Y. Loh**. 1988. A Conserved Sequence in the T-Cell Receptor β-Chain Promoter Region. *Proc. Natl. Acad. Sci. USA. 85:3551.*

111. **Diamond, D. J., F. B. Nelson, and E. L. Reinherz**. 1989. Lineage-Specific Expression of a T Cell Receptor Variable Gene Promoter Controlled by Upstream Sequences. *J. Exp. Med. 169:1213.*

112. **Anderson, S. J., S. Miyake, and D. Y. Loh**. 1989. Transcription from a Murine T-Cell Receptor Vβ Promoter Depends on a Conserved Decamer Motif Similar to the Cyclic AMP Response Element. *Mol. Cell. Biol. 9:4835.*

113. **Ratanavongsiri, J., S. Igarashi, S. Mangal, P. Kilgannon, A. Fu, and A. Fotedar**. 1990. Transcription of the T Cell Receptor β-Chain Gene is Controlled by Multiple Regulatory Elements. *J. Immunol. 144:1111.*

114. **Li, Y., P. Szabo, and D. N. Posnett**. 1991. The Genomic Structure of Human Vβ6 T Cell Antigen Receptor Genes. *J. Exp. Med. 174:1537.*

115. **Lee, M.-R., C.-S. Chung, M.-L. Liou, M. Wu, W.-F. Li, Y.-P. Hsueh, and M.-Z. Lai**. 1992. Isolation and Characterization of Nuclear Proteins that Bind to T Cell Receptor Vβ Decamer Motif. *J. Immunol. 148:1906.*

116. **Bier, E., Y. Hashimoto, M. I. Greene, and A. M. Maxam**. 1985. Active T-Cell Receptor Genes Have Intron Deoxyribonuclease Hypersensitive Sites. *Science 229:528.*

117. **Bories, J. C., P. Loiseau, L. d'Auriol, C. Gontier, A. Bensussan, L. Degos, and F. Sigaux**. 1990. Regulation of Transcription of the Human T Cell Antigen Receptor δ Chain Gene. A T Lineage-Specific Enhancer Element is Located in the Jδ3-Cδ Intron. *J. Exp. Med. 171:75.*

118. **Hashimoto, Y**. 1989. T Cell Receptor β Gene has Two Downstream DNase I Hypersensitive Regions. Possible Mechanisms of Tissue- and Stage-Specific Gene Regulation. *J. Exp. Med. 169:2097.*

119. **Mauxion, F., M. G. Pray, and R. Sen**. 1990. Multiple Nuclear Factors Interact with Sequences within the Jβ2-Cβ2 Intron of the Murine T Cell Receptor β-Chain Gene. *J. Immunol. 145:1577.*

120. **Jamieson, C., F. Mauxion, and R. Sen**. 1989. Identification of a Functional NF-kB Binding Site in the Murine T-Cell Receptor β2 Locus. *J. Exp. Med. 170:1737.*

121. **Oosterwegel, M., M. van de Wetering, D. Dooijes, L. Klomp, A. Winoto, K. Georgopoulos, F. Meijlink, and H. Clevers**. 1991. Cloning of Murine TCF-1, a T Cell-Specific Transcription Factor Interacting with Functional Motifs in the CD3-ϵ and T Cell Receptor α Enhancers. *J. Exp. Med. 173:1133.*

122. **van de Wetering, M., M. Oosterwegel, D. Dooijes, and H. Clevers**. 1991. Identification and Cloning of TCF-1, a T Lymphocyte-Specific Transcription Factor Containing a Sequence-Specific HMG Box. *EMBO J. 10:123.*

123. **Travis, A., A. Amsterdam, C. Belanger, and R. Grosschedl**. 1991. LEF-1, A Gene Encoding a Lymphoid-Specific Protein with an HMG Domain, Regulates T-Cell Receptor α Enhancer Function. *Genes Dev. 5:880.*

124. **Ho, I.-C., N. K. Bhat, L. R. Gottschalk, T. Lindsten, C. B. Thompson, T. S. Papas, and J. M. Leiden**. 1990. Sequence-Specific Binding of Human Ets-1 to the T Cell Receptor α Gene Enhancer. *Science 250:814.*

125. **Bhat, N. K., K. L. Komschlies, S. Fujiwara, R. J. Fisher, B. J. Mathieson, T. A. Gregorio, H. A. Young, J. W. Kasik, K. Ozato, and T. S. Papas**. 1989. Expression of *ets* Genes in Mouse Thymocyte Subsets and T Cells. *J. Immunol. 142:672.*

126. **Ho, I.-C., and J. M. Leiden**. 1990. The Tα2 Nuclear protein Binding Site from the Human T Cell Receptor α Enhancer Functions as Both a T Cell-Specific Transcriptional Activator and Repressor. *J. Exp. Med. 172:1443.*

127. **Ho, I.-C., P. Vorhees, N. Marin, B. K. Oakley, S.-F. Tsai, S. H. Orkin, and J. M. Leiden**. 1991. Human GATA-3: A Lineage-Restricted Transcription Factor that Regulates the Expression of the T Cell Receptor α Gene. *EMBO J. 10:1187.*

128. **Marine, J., and A. Winoto**. 1991. The Human Enhancer-Binding Protein Gata3 Binds to Several T-Cell Receptor Regulatory Elements. *Proc. Natl. Acad. Sci. USA. 88:7284.*

129. **Martin, D. I. K., L. I. Zon, G. Mutter, and S. H. Orkin**. 1990. Expression of an Erythroid Transcription Factor in Megakaryocytic and Mast Cell Lineages. *Nature 344:444.*

130. **Romeo, P.-H., M.-H. Prandini, V. Joulin, V. Mignotte, M. Prenant, W. Vainchenker, G. Marguerie, and G. Uzan**. 1990. Megakaryocytic and Erythrocytic Lineages Share Specific Transcription Factors. *Nature 344:447.*

131. **Pevny, L., M. C. Simon, E. Robertson, W. H. Klein, S.-F. Tsai, V. D'Agati, S. H. Orkin, and F. Costantini**. 1991. Erythroid Differentiationin Chimaeric Mice Blocked by a Targeted Mutation in the Gene for Transcription Factor GATA-1. *Nature 349:257.*

132. **Yamamoto, M., L. J. Ko, M. W. Leonard, H. Beug, S. H. Orkin, and J. D. Engel**. 1990. Activity and Tissue-Specific Expression of the Transcription Factor NF-E1 Multigene Family. *Genes Dev. 4:1650.*

133. **Ko, L. J., M. Yamamoto, M. W. Leonard, K. M. George, P. Ting, and J. D. Engel**. 1991. Murine and Human T-Lymphocyte GATA-3 Factors Mediate Transcription Through a *cis*-Regulatory Element within the Human T-Cell Receptor δ Gene Enhancer. *Mol. Cell. Biol. 11:2778.*

134. **Joulin, V., D. Bories, J.-F. Eléouet, M.-C. Labastie, S. Chrétien, M.-G. Mattéi, and P.-H. Roméo**. 1991. A T-Cell Specific TCR δ DNA Binding Protein is a Member of the Human GATA Family. *EMBO J. 10:1809.*

135. **Krimpenfort, P., R. de Jong, Y. Uematsu, Z. Dembic, S. Ryser, H. von Boehmer, M. Steinmetz, and A. Berns**. 1988. Transcription of T Cell Receptor β-Chain Gene is Controlled by a Downstream Regulatory Element. *EMBO J. 7:745.*

136. **Takeda, J., A. Cheng, F. Mauxion, C. A. Nelson, R. D. Newberry, W. C. Sha, R. Sen, and D. Y. Loh**. 1990. Functional Analysis of the Murine T-Cell Receptor β Enhancer and Characteristics of its DNA-Binding Proteins. *Mol. Cell. Biol. 10:5027.*

137. **Halloran, P. F., and J. Madrenas**. 1990. Regulation of MHC Transcription.

Transplantation 50:725.

138. **Gottschalk, L. R., and J. M. Leiden**. 1990. Identification and Functional Characterization of the Human T-Cell Receptor β Gene Transcriptional Enhancer: Common Nuclear Proteins Interact with the Transcriptional Regulatory Elements of the T-Cell receptor α and β Genes. *Mol. Cell. Biol. 10:5486.*

139. **MacLeod, C. L., L. Minning, D. P. Gold, C. Terhorst, and M. Wilkinson**. 1986. Negative Trans-Regulation of T-Cell Antigen Receptor/T3 Complex mRNA Expression in Murine T-Lymphoma Somatic Cell Hybrids. *Proc. Natl. Acad. Sci. USA. 83:6989.*

140. **Wilkinson, M. F., and C. L. MacLeod**. 1988. Induction of T-Cell Receptor-α and -β mRNA in SL12 Cells can Occur by Transcriptional and Post-Transcriptional Mechanisms. *EMBO J. 7:101.*

141. **Lindsten, T., C. H. June, and C. B. Thompson**. 1988. Transcription of T Cell Antigen Receptor Genes is Induced by Protein Kinase C Activation. *J. Immunol. 141:1769.*

142. **Martinez-Valdez, H., P. J. Doherty, E. Thompson, S. H. Benedict, E. W. Gelfand, and A. Cohen**. 1988. Antagonistic Effects of Calcium Ionophores and Phorbol Esters on T Cell Receptor mRNA Levels in Human Thymocytes. *J. Immunol. 140:361.*

143. **Spencer, D. M., Y.-H. Hsiang, J. P. Goldman, and D. H. Raulet**. 1991. Identification of a T-Cell-Specific Transcriptional Enhancer Located 3' of Cγ1 in the Murine T-Cell Receptor γ Locus. *Proc. Natl. Acad. Sci. USA. 88:800.*

144. **Weinstein, Y., K. Morishita, J. L. Cleveland, and J. N. Ihle**. 1989. Interleukin 3 (IL-3) Induces Transcription from Nonrearranged T Cell Receptor γ Loci in IL-3-Dependent Cell Lines. *J. Exp. Med. 169:2059.*

145. **Miyajima, A., S. Miyatake, J. Schreurs, J. de Vries, N. Arai, T. Yokota, and K.-I. Arai**. 1988. Coordinate Regulation of Immune and Inflammatory Responses by T Cell-Derived Lymphokines. *FASEB J. 2:2462.*

146. **Gauchat, J.-F., D. A. Lebman, R. L. Coffman, H. Gascan, and J. E. de Vries**. 1990. Structure and Expression of Germline ε Transcripts in Human B Cells induced by IL-4 to Switch IgE Production. *J. Exp. Med. 172:463.*

147. **Shockett, P., and J. Stavnezer**. 1991. Effect of Cytokines on Switching to IgA and α Germline Transcripts in the B Cell Lymphoma I.29μ. Transforming Growth Factor-β Activates Transcription of the Unrearranged C_α Gene. *J. Immunol. 147:4374.*

148. **Redondo, J. M., S. Hata, C. Brocklehurst, and M. S. Krangel**. 1990. A T Cell-Specific Transcriptional Enhancer within the Human T Cell Receptor δ Locus. *Science 247:1225.*

149. **Gill, L. L., D. Zaninetta, and K. Karjalainen**. 1991. A Transcriptional Enhancer of the Mouse T Cell Receptor δ Gene Locus. *Eur. J. Immunol. 21:807.*

150. **Redondo, J. M., J. L. Pfohl, and M. S. Krangel**. 1991. Identification of an Essential Site for Transcriptional Activation within the Human T-Cell Receptor δ Enhancer. *Mol. Cell. Biol. 11:5671.*

151. **Winoto, A., and D. Baltimore**. 1989. $\alpha\beta$ Lineage-Specific Expression of the α T Cell Receptor Gene by Nearby Silencers. *Cell 59:649.*

152. **Ishida, I., S. Verbeek, M. Bonneville, S. Itohara, A. Berns, and S. Tonegawa**. 1990. T Cell Receptor $\gamma\delta$ and γ Transgenic Mice Suggest a Role of a γ Gene Silencer in the Generation of $\alpha\beta$ T Cells. *Proc. Natl. Acad. Sci. USA. 87:3067.*

153. **Bonneville, M., I. Ishida, P. Mombaerts, M. Katsuki, S. Verbeek, A. Berns, and S. Tonegawa**. 1989. Blockade of $\alpha\beta$ T-Cell Development by TCR $\gamma\delta$ Transgenes. *Nature 342:931.*

154. **Winoto, A., and D. Baltimore**. 1989. Separate Lineages of T Cells Expressing the $\alpha\beta$ and $\gamma\delta$ Receptors. *Nature 338:430.*

155. **Takeshita, S., M. Toda, and H. Yamagishi**. 1989. Excision Products of the T Cell Receptor Gene Support a Progressive Rearrangemnt Model of the α/δ Locus. *EMBO J. 8:3261.*

156. **Manolios, N., F. Letourneur, J. S. Bonifacino, and R. D. Klausner**. 1991. Pairwise, Cooperative and Inhibitory Interactions Describe the Assembly and Probable Structure of the T-Cell Antigen Receptor. *EMBO J. 10:1643.*

157. **Hue, I., J. Trucy, C. McCoy, D. Couez, B. Malissen, and M. Malissen**. 1990. A Novel Type of Aberrant T Cell Receptor α-Chain Gene Rearrangement. Implications for Allelic Exclusion and the V-J Recombination Process. *J. Immunol. 144:4410.*

158. **Blüthmann, H., P. Kisielow, Y. Uematsu, M. Malissen, P. Krimpenfort, A. Berns, H. von Boehmer, and M. Steinmetz**. 1988. T-Cell-Specific Deletion of T-Cell Receptor Transgenes Allows Functional Rearrangement of Endogenous α- and β-Genes. *Nature 334:156.*

159. **Uematsu, Y., S. Ryser, Z. Dembic, P. Borgulya, P. Krimpenfort, A. Berns, H. von Boehmer, and M. Steinmetz**. 1988. In Transgenic Mice the Introduced Functional T Cell Receptor β Gene Prevents Expression of Endogenous Genes. *Cell 52:831.*

160. **Becker, M. L. B., R. Near, M. Mudgett-Hunter, M. N. Margolies, R. T. Kubo, J. Kaye, and S. M. Hedrick**. 1989. Expression of a Hybrid Immunoglobulin-T Cell Receptor Protein in Transgenic Mice. *Cell 58:911.*

161. **Krimpenfort, P., F. Ossendorp, J. Borst, C. Melief, and A. Berns**. 1989. T Cell Depletion in Transgenic Mice Carrying a Mutant Gene for TCR-β. *Nature 341:742.*

162. **van Meerwijk, J. P. M., H. Blüthmann, and M. Steinmetz**. 1990. T-Cell Specific Rearrangement of T-Cell Receptor β Transgenes in Mice. *EMBO J. 9:1057.*

163. **van Meerwijk, J. P. M., A. Iglesias, T. Hansen-Hagge, H. Bluethmann, and M. Steinmetz**. 1991. Allelic Exclusion of a T Cell Receptor-β Minilocus. *J. Immunol. 147:3224.*

164. **Berg, L. J., A. M. Pullen, B. Fazekas de St. Groth, D. Mathis, C. Benoist, and M. M. Davis**. 1989. Antigen/MHC-Specific T Cells Are Preferentially Exported from the Thymus in the Presence of their MHC Ligand. *Cell 58:1035.*

165. **Kisielow, P., H. Blüthmann, U. D. Staerz, M. Steinmetz, and H. von Boehmer**. 1988. Tolerance in T-Cell-Receptor Transgenic Mice Involves Deletion of Nonmature CD4$^+$8$^+$ Thymocytes. *Nature 333:742.*

166. **Kaye, J., M. L. Hsu, M. E. Sauron, S. C. Jameson, N. R. J. Gascoigne, and S. M. Hedrick**. 1989. Selective Development of CD4$^+$ T Cells in Transgenic Mice Expressing a Class II MHC-Restricted Antigen Receptor. *Nature 341:746.*

167. **Borgulya, P., H. Kishi, Y. Uematsu, and H. von Boehmer**. 1992. Exclusion and Inclusion of α and β T Cell Receptor Alleles. *Cell 69:529.*

168. **Paillard, F., G. Sterkers, and C. Vaquero**. 1990. Transcriptional and Post-Transcriptional Regulation of TcR, CD4 and CD8 Gene Expression During Activation of Normal Human T Lymphocytes. *EMBO J. 9:1867.*

169. **Schönrich, G., U. Kalinke, F. Momburg, M. Malissen, A.-M. Schmitt-Verhulst, B. Malissen, G. J. Hämmerling, and B. Arnold**. 1991. Down-Regulation of T Cell Receptors on Self-Reactive T Cells as a Novel Mechanism for Extrathymic Tolerance Induction. *Cell 65:293.*

170. **Fowlkes, B. J., R. H. Schwartz, and D. M. Pardoll**. 1988. Deletion of Self-Reactive Thymocytes Occurs at a CD4$^+$8$^+$Precursor Stage. *Nature 334:620.*

171. **Bonifacino, J. S., S. A. McCarthy, J. E. Maguire, T. Nakayama, D. S. Singer, R. D. Klausner, and A. Singer**. 1990. Novel Post-Translational Regulation of TCR Expression in CD4$^+$CD8$^+$ Thymocytes Influenced by CD4. *Nature 344:247.*

172. **Weissman, A. M., S. J. Frank, D. J. Orloff, M. Mercep, J. D. Ashwell, and R. D. Klausner**. 1989. Role of the Zeta Chain in the Expression of the T Cell

Antigen Receptor: Genetic Reconstitution Studies. *EMBO J. 8:3651.*

173. **Madrenas, J., N. A. Parfrey, and P. F. Halloran**. 1991. Interferon Gamma-Mediated Renal MHC Expression in Mercuric Chloride-Induced Glomerulonephritis. *Kidney Int. 39:273.*

174. **Schreiner, G. F., K. P. G. Harris, M. L. Purkerson, and S. Klahr**. 1988. Immunological Aspects of Acute Ureteral Obstruction: Immune Cell Infiltrate in the Kidney. *Kidney Int. 34:487.*

175. **Sambrook, J., E. F. Fritch, and T. Maniatis**. 1989. *Molecular Cloning: a Laboratory Manual*. 2nd edition. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

176. **Chomczynski, P., and N. Sacchi**. 1987. Single-Step Method of RNA Isolation by Acid Guanidinium Thiocyanate-Phenol-Chloroform Extraction. *Anal. Biochem. 162:156.*

177. **Bothwell, A., G. D. Yancopoulos, and F. W. Alt**. 1990. *Methods for Cloning and Analysis of Eukaryotic Genes*. Jones and Bartlett Publishers, Boston.

178. **Iwamoto, A., P. S. Ohashi, H. Pircher, C. L. Walker, E. E. Michalopoulos, F. Rupp, H. Hengartner, and T. W. Mak**. 1987. T Cell Receptor Variable Gene Usage in a specific Cytotoxic T Cell Response. Primary Structure of the Antigen-MHC Receptor of Four Hapten-Specific Cytotoxic T Cell Clones. *J. Exp. Med. 165:591.*

179. **Caccia, N., M. Kronenberg, D. Saxe, R. Haars, G. Bruns, J. Goverman, M. Malissen, H. Willard, Y. Yoshikai, M. Simon, L. Hood, and T. Mak**. 1984.

The T Cell Receptor β Chain Genes are Located on Chromosome 6 in Mice and Chromosome 7 in Humans. *Cell 37:1091.*

180. **Ferrick, D. A., S. R. Sambhara, W. Ballhausen, A. Iwamoto, H. Pircher, C. L. Walker, W. M. Yokohama, R. G. Miller, and T. W. Mak.** 1989. T Cell Function and Expression are Dramatically Altered in T Cell Receptor Vγ1.1Jγ4Cγ4 Transgenic Mice. *Cell 57:483.*

181. **Davis, M. M., D. I. Cohen, E. A. Nielsen, M. Steinmetz, W. E. Paul, and L. Hood**. 1984. Cell-Type-Specific cDNA Probes and the Murine I Region: The Localization and Orientation of Aαd. *Proc. Natl. Acad. Sci. USA. 81:2194.*

182. **Parnes, J. R., and J. G. Seldman**. 1982. Structure of wild-type and mutant β_2-microglobulin genes. *Cell 29:661.*

183. **Chien, Y., D. M. Becker, T. Lindsten, M. Okamura, D. I. Cohen, and M. M. Davis**. 1984. A Third Type of Murine T-Cell Receptor Gene. *Nature 312:31.*

184. **Cleveland, D. W., M. A. Lopata, R. J. MacDonald, N. J. Cowan, W. J. Rutter, and M. W. Kirschner**. 1980. Number and Evolutionary Conservation of α- and β-Tubulin and Cytoplasmic β- and γ- Actin Genes Using Specific Cloned cDNA Probes. *Cell 20:95.*

185. **Kawasaki, E. S**. 1990. Amplification of RNA. In *PCR Protocols. A Guide to Methods and Applications.* M. A. Innis, D. H. Gelfand, J. J. Sninsky and T. J. White. Academic Press, Inc., San Diego, p. 21.

186. **Hayday, A. C., D. J. Diamond, G. Tanigawa, J. S. Heilig, V. Folsom, H. Saito, and S. Tonegawa**. 1985. Unusual Organization and Diversity of T-Cell

Receptor α-Chain Genes. *Nature 316:828.*

187. **Glimcher, L., F. W. Shen, and H. Cantor**. 1977. Identification of a Cell Surface Antigen Selectively Expressed on the Natural Killer Cell. *J. Exp. Med. 145:1.*

188. **Oi, V. T., P. P. Jones, J. W. Goding, and L. A. Herzenberg**. 1978. Properties of Monoclonal Antibodies to Mouse Ig Allotypes, H-2, and Ia Antigens. *Curr. Top. Microbiol. Immunol. 81:115.*

189. **Springer, T., G. Galfre, D. S. Secher, and C. Milstein**. 1979. Mac-1: A Macrophage Differentiation Antigen Identified by Monoclonal Antibody. *Eur. J. Immunol. 9:301.*

190. **Leo, O., M. Foo, D. H. Sachs, L. E. Samelson, and J. A. Bluestone**. 1987. Identification of a Monoclonal Antibody Specific for a Murine T3 Polypeptide. *Proc. Natl. Acad. Sci. USA. 84:1374.*

191. **Ledbetter, J. A., and L. A. Herzenberg**. 1979. Xenogeneic Monoclonal Antibodies to Mouse Lymphoid Differentiation Antigens. *Immunol. Rev. 47:63.*

192. **Gomez, L. J., I. D. Walker, M. S. Sandrin, and I. F. C. McKenzie**. 1987. High Sequence Conservation Between Rat (T200) and Mouse (Ly-5) Leukocyte Common Antigens. *Immunogenetics 25:263.*

193. **Gnann, J. W. J., J. Ahimen, C. Svalander, L. Olding, M. B. A. Oldstone, and J. A. Nelson**. 1988. Inflammatory Cells in Transplanted Kidneys are Infected by Human Cytomegalovirus. *Am. J. Pathol. 132:239.*

194. **Gilman, M**. 1991. Ribonuclease Protection Assay. In *Current Protocols in*

Molecular Biology. F. M. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith and K. Struhl. John Wiley & Sons, New York, p. 4.

195. **Madrenas, J., F. Pazderka, N. A. Parfrey, and P. F. Halloran**. 1992. Thymus-Independent Expression of a Truncated T Cell Receptor-α mRNA in Murine Kidney. *J. Immunol. 148:612.*

196. **Springer, T. A**. 1991. Isolation of Proteins Using Antibodies. In *Current Protocols in Immunology.* J. E. Coligan, A. M. Kruisbeek, D. H. Margulies, E. M. Shevach and W. Strober. John Wiley & Sons, New York, p. 8.

197. **Shultz, L. D., and C. L. Sidman**. 1987. Genetically Determined Murine Models of Immunodeficiency. *Annu. Rev. Immunol. 5:367.*

198. **Loh, E. Y., J. F. Elliott, S. Cwirla, L. L. Lanier, and M. M. Davis**. 1989. Polymerase Chain Reaction with Single-Sided Specificity: Analysis of T Cell Receptor δ Chain. *Science 243:217.*

199. **Gu, H., D. Kitamura, and K. Rajewsky**. 1991. B Cell Development Regulated by Gene Rearrangement: Arrest of Maturation by Membrane-Bound D_{μ} Protein and Selection of D_H Element Reading Frames. *Cell 65:47.*

200. **Berke, G**. 1989. Functions and Mechanisms of Lysis Induced by Cytotoxic T Lymphocytes and Natural Killer Cells. In *Fundamental Immunology. 2nd.* W. E. Paul. Raven Press, New York, p. 735.

201. **Anderson, P., M. Caligiuri, J. Ritz, and S. F. Schlossman**. 1989. CD3-Negative Natural Killer Cells Express ζ TCR as Part of a Novel Molecular Complex. *Nature 341:159.*

202. **Young, H. A., J. R. Ortaldo, R. B. Herberman, and C. W. Reynolds.** 1986. Analysis of T Cell Receptors in Highly Purified Rat and Human Large Granular Lymphocytes (LGL): Lack of Functional 1.3 kb β-Chain mRNA. *J. Immunol. 136:2701.*

203. **Pelicci, P. G., P. Allavena, M. Subar, A. Rambaldi, A. Pirelli, M. Di Bello, T. Barbui, D. M. Knowles II, R. Dalla-Favera, and A. Mantovani.** 1987. T Cell Receptor (α, β, γ) Gene Rearrangement and Expression in Normal and Leukemic Large Granular Lymphocytes/Natural Killer Cells. *Blood 70:1500.*

204. **Roman-Roman, S., E. Baixeras, C. Genevee, T. Hercend, and F. Thriebel.** 1989. The T-Cell Receptor Vδ Genes Predominantly Used by Human Peripheral γ/δ^+ T Lymphocytes are not Rearranged in CD3$^-$ Natural Killer Cells. *Human Immunol. 26:75.*

205. **Biondi, A., P. Allavena, V. Rossi, R. Bassan, T. Barbui, E. Champagne, T. W. Mak, M. D. Minden, A. Rambaldi, and A. Mantovani.** 1989. T Cell Receptor δ Gene Organization and Expression in Normal and Leukemic Natural Killer Cells. *J. Immunol. 143:1009.*

206. **Lemley, K. V., and W. Kriz.** 1991. Anatomy of the Renal Interstitium. *Kidney Int. 39:370.*

207. **Neilson, E. G.** 1989. Pathogenesis and Therapy of Interstitial Nephritis. *Kidney Int. 35:1257.*

208. **Bulger, R. E., and R. B. Nagle.** 1973. Ultrastructure of the Interstitium in the Rabbit Kidney. *Am. J. Anat. 136:183.*

209. **Bulger, R. E., C. C. Tisher, C. H. Myers, and B. F. Trump**. 1967. Human Renal Ultrastructure. II. The Thin Limb of Henle's Loop and the Interstitium in Healthy Individuals. *Lab. Invest. 16:124.*

210. **Bohman, S.-O**. 1974. The Ultrastructure of the Rat Renal Medulla as Observed after Improved Fixation Methods. *J. Ultrastruct. Res. 47:329.*

211. **Ekblom, P., and A. Weller**. 1991. Ontogeny of Tubulointerstitial Cells. *Kidney Int. 39:394.*

212. **Hart, D. N. J., and J. W. Fabre**. 1981. Demonstration and Characterization of Ia-Positive Dendritic Cells in the Interstitial Connective Tissues of Rat Heart and Other Tissues, but not Brain. *J. Exp. Med. 154:347.*

213. **Halloran, P. F., P. Autenreid, and A. Wadgymar**. 1986. Regulation of HLA Antigen Expression in Human Kidney. *Clinics in Immunol. and Allergy 6:411.*

214. **Bishop, G. A., and B. M. Hall**. 1989. Expression of Leukocyte and Lymphocyte Adhesion Molecules in the Human Kidney. *Kidney Int. 36:1078.*

215. **Steinman, R. M., and M. C. Nussenzweig**. 1980. Dendritic Cells: Features and Functions. *Immunol. Rev. 53:127.*

216. **Steinman, R. M**. 1991. The Dendritic Cell System. *Annu. Rev. Immunol. 9:271.*

217. **Austyn, J. M**. 1987. Lymphoid Dendritic Cells. *Immunol. 62:161.*

218. **Unanue, E. R**. 1989. Macrophages, Antigen-Presenting Cells, and the Phenomena of Antigen Handling and Presentation. In *Fundamental Immunology. 2nd.* W. E. Paul. Raven Press, New York, p. 95.

219. **Hart, D. N., and J. W. Fabre**. 1981. Endogenously Produced Ia Antigens within Cells of Convoluted Tubules of Rat Kidney. *J. Immunol. 126:2109.*

220. **Hart, D. N. J., and J. W. Fabre**. 1981. Major Histocompatibility Complex Antigens in Rat Kidney, Ureter, and Bladder. *Transplantation 31:318.*

221. **Mayrhofer, G., and M. A. Schon-Hegrad**. 1983. Ia Antigens in Rat Kidney, with Special Reference to their Expression in Tubular Epithelium. *J. Exp. Med. 157:2097.*

222. **Steiniger, B., J. Klempnauer, and K. Wonigeit**. 1984. Phenotype and Histological Distribution of Interstitial Cells in the Rat Pancreas, Liver, Heart, and Kidney. *Transplantation 38:169.*

223. **Pollard, A. M., and M. F. Lipscomb**. 1990. Characterization of Murine Lung Dendritic Cells: Similarities to Langerhans Cells and Thymic Dendritic Cells. *J. Exp. Med. 172:159.*

224. **Crowley, M., K. Inaba, M. Witmer-Pack, and R. M. Steinman**. 1989. The Cell Surface of Mouse Dendritic Cells: FACS Analyses of Dendritic Cells from Different Tissues Including Thymus. *Cell. Immunol. 118:108.*

225. **Roitt, I., J. Brostoff, and D. Male**. 1985. *Immunology*. The C.V. Mosby Company, London.

226. **Shevach, E. M**. 1989. Accessory Molecules. In *Fundamental Immunology. 2nd.* W. E. Paul. Raven Press, New York, p. 413.

227. **Palacios, R., M. Kiefer, M. Brockhaus, K. Karjalainen, Z. Dembic, P. Kisielow, and H. von Boehmer**. 1987. Molecular, Cellular, and Functional

Properties of Bone Marrow T Lymphocyte Progenitor Clones. *J. Exp. Med. 166:12.*

228. **Havran, W. L., M. Poenie, R. E. Tigelaar, R. Y. Tsien, and J. P. Allison**. 1989. Phenotypic and Functional Analysis of γ/δ T Cell Receptor-Positive Murine Dendritic Epidermal Clones. *J. Immunol. 142:1422.*

229. **Nixon-Fulton, J. L., W. A. Kuziel, B. Santerse, P. R. Bergstresser, P. W. Tucker, and R. E. Tigelaar**. 1988. Thy-1$^+$ Epidermal Cells in Nude Mice are Distinct from Their Counterparts in Thymus-Bearing Mice. A Study of Morphology, Function, and T Cell Receptor Expression. *J. Immunol. 141:1897.*

230. **Hurwitz, J. L., J. Samaridis, and J. Pelkonen**. 1988. Progression of Rearrangements at T Cell Receptor β And γ Gene Loci during Athymic Differentiation of Bone Marrow Cells In Vitro. *Cell 52:821.*

231. **Speiser, D. E., U. Stübi, and R. M. Zinkernagel**. 1992. Extrathymic Positive Selection of αß T-Cell Precursors in Nude Mice. *Nature 355:170.*

232. **MacDonald, H. R., R. K. Lees, C. Bron, B. Sordat, and G. Miescher**. 1987. T Cell Antigen Receptor Expression in Athymic (nu/nu) Mice. *J. Exp. Med. 166:195.*

233. **Saito, H., D. M. Kranz, Y. Takagaki, A. C. Hayday, H. N. Eisen, and S. Tonegawa**. 1984. A Third Rearranged and Expressed Gene in a Clone of Cytotoxic T Lymphocytes. *Nature 312:36.*

234. **Arden, B., J. L. Klotz, G. Siu, and L. E. Hood**. 1985. Diversity and Structure of Genes of the α Family of Mouse T-Cell Antigen Receptor. *Nature 316:783.*

235. **Berman, J. E., C. G. Humphries, J. Barth, F. W. Alt, and P. W. Tucker.** 1991. Structure and Expression of Human Germline V_H Transcripts. *J. Exp. Med. 173:1529.*

236. **Lutzker, S., and F. W. Alt.** 1988. Structure and Expression of Germline Immunoglobulin γ2b Transcripts. *Mol. Cell. Biol. 8:1849.*

237. **Sideras, P., T.-R. Mizuta, H. Kanamori, N. Suzuki, M. Okamoto, K. Kuze, H. Ohno, S. Doi, S. Fukuhara, M. S. Hassan, L. Hammarstrom, E. Smith, A. Shimizu, and T. Honjo.** 1989. Production of Sterile Transcripts of C_γ Genes in an IgM Producing Human Neoplastic B Cell Line that Switches to IgG Producing Cells. *Int. Immunol. 1:631.*

238. **Carroll, A. M., and M. J. Bosma.** 1991. T-Lymphocyte development in scid Mice is Arrested Shortly after the Initiation of T-Cell Receptor δ Gene Recombination. *Genes Dev. 5:1357.*

239. **Bosma, M. J., and A. M. Carroll.** 1991. The SCID Mouse Mutant: Definition, Characterization, and Potential Uses. *Annu. Rev. Immunol. 9:323.*

240. **Collier, D. A., J. A. Griffin, and R. D. Wells.** 1988. Non-B Right-Handed DNA Conformations of Homopurine-Homopyrimidine Sequences in the Murine Immunoglobulin Cα Switch Region. *J. Biol. Chem. 263:397.*

241. **Tanaka, T., C. C. Chu, and W. E. Paul.** 1992. An Antisense Oligonucleotide Complementary to a Sequence in Iγ2b Increases γ2b Germline Transcripts, Stimulates B Cell DNA Synthesis, and Inhibits Immunoglobulin Secretion. *J. Exp. Med. 175:597.*

242. **Shimizu, A., M. C. Nussenzweig, H. Han, M. Sanchez, and T. Honjo**. 1991. *Trans*-Splicing as a Possible Molecular Mechanism for the Multiple Isotype Expression of the Immunoglobulin Gene. *J. Exp. Med. 173:1385.*

243. **Han, H., M. Okamoto, T. Honjo, and A. Shimizu**. 1991. Regulated Expression of Immunoglobulin *Trans*-mRNA Consisting of the Variable Region of a Transgenic μ Chain and Constant Regions of Endogenous Isotypes. *Int. Immunol. 3:1197.*

244. **Zheng, H., M. Boyer, A. Fotedar, B. Singh, and D. R. Green**. 1988. An Antigen-Specific Helper T Cell Hybridoma Produces an Antigen-Specific Suppressor Inducer Molecule with Identical Antigenic Fine Specificity. Implications for the Antigen Recognition and Function of Helper and Suppressor Inducer T Cells. *J. Immunol. 140:1351.*

245. **Zheng, H., B. M. Sahai, P. Kilgannon, A. Fotedar, and D. R. Green**. 1989. Specific Inhibition of Cell-Surface T-Cell Receptor Expression by Antisense Oligodeoxynucleotides and Its Effect on the Production of an Antigen-Specific Regulatory T-Cell Factor. *Proc. Natl. Acad. Sci. USA. 86:3758.*

246. **Iwata, M., K. Katamura, R. T. Kubo, and K. Ishizaka**. 1989. Relationship between T Cell Receptors and Antigen-Binding Factors. I. Specificity of Functional T Cell Receptors on Mouse T Cell Hybridomas that Produce Antigen-Binding T Cell Factors. *J. Immunol. 143:3909.*

247. **Iwata, M., K. Katamura, R. T. Kubo, H. M. Grey, and K. Ishizaka**. 1989. Relationship between T Cell Receptors and Antigen-Binding Factors. II. Common Antigenic Determinants and Epitope Recognition Shared by T Cell Receptors and Antigen-Binding Factors. *J. Immunol. 143:3917.*

248. **Bissonnette, R., H. Zheng, R. T. Kubo, B. Singh, and D. R. Green**. 1991. A T Helper Cell Hybridoma Produces an Antigen-Specific Regulatory Activity. Relationship to the T Cell Receptor by Serology and Antigenic Fine Specificity. *J. Immunol. 146:2898.*

249. **Green, D. R., R. Bissonnette, H. Zheng, T. Onda, F. Echeverri, R. J. Mogil, J. K. Steele, M. Voralia, and A. Fotedar**. 1991. Immunoregulatory Activity of the T-Cell Receptor α Chain Demonstrated by Retroviral Gene Transfer. *Proc. Natl. Acad. Sci. USA. 88:8475.*

250. **Kuchroo, V. K., M. C. Byrne, Y. Atsumi, E. Greenfield, J. B. Connolly, M. J. Whitters, R. M. J. O'Hara, M. Collins, and M. E. Dorf**. 1991. T-Cell Receptor α Chain Plays a Critical Role in Antigen-Specific Suppressor Cell Function. *Proc. Natl. Acad. Sci. USA. 88:8700.*

251. **Kang, J., J. Wither, and N. Hozumi**. 1990. Long-Term Expression of a T-Cell Receptor β-Chain Gene in Mice Reconstituted with Retrovirus-Infected Hematopoietic Stem Cells. *Proc. Natl. Acad. Sci. USA. 87:9803.*

252. **Morley, B.J., K.N. Chin, M.E. Newton, and A. Weiss**. 1988. The Lysine Residue in the Transmembrane-Spanning Domain of the ß Chain is Necessary for Cell Surface Expression of the T Cell Antigen Receptor. *J. Exp. Med. 168:1971.*

253. **Alcover, A., R.A. Mariuzza, M. Ermonval, and O. Acuto**. 1990. Lysine 271 in the Transmembrane Domain of the T-Cell Antigen Receptor ß Chain is Necessary for its Assembly with the CD3 Complex but not α/ß Dimerization. *J. Biol. Chem. 265:4131.*

254. **Engel, I., and S.M. Hedrick**. 1988. Site-Directed Mutations in the VDJ Junctional Region of a T Cell Receptor ß Chain Cause Changes in Antigenic Peptide Recognition. *Cell 31:473.*

255. **Danska, J.S., A.M. Livingstone, V. Paragas, T. Ishihara, and C.G. Fathman**. 1990. The Presumptive CDR3 Regions of both T Cell Receptor α and ß Chains Determine T Cell Specificity for Myoglobin Peptides. *J. Exp. Med. 172:27.*

256. **Maguire J.E., S.A. McCarthy, A. Singer, and D.S. Singer**. 1990. Inverse Correlation Between Steady-State RNA and Cell Surface T Cell Receptor Levels. *FASEB J. 4:3131.*

APPENDIX I:

CHARACTERIZATION OF THE 5' END OF THE TRUNCATED TCR α mRNA

Since the completion of this thesis, and as it was stated in the "Future Perspectives" Section, a major effort has been focused on the characterization of the 5' end of the truncated TCR α mRNA. The difficulties encountered in this task were attributed to the ability of the C region of the truncated TCR α transcript to form at least two secondary structures. These structures would minimize the efficiency of reverse transcription by stopping the reverse transcriptase. Considering that the transcript is expressed in the kidney in lower amounts than in lymphoid organs, the low efficiency of the reverse transcription reaction would lead to a poor representation of the truncated TCR α transcript in cDNA libraries. Based on this argument, we decided to approach the problem using anchored PCR.

The technique of anchored PCR was originally described to characterize the repertoire of the TCR δ chain in human PBL (198). The technique involves reverse transcription of the target sequence followed by the separation of the cDNA from oligoprimers, tailing of the cDNA with d(G), and amplification of the cDNA with an specific oligoprimer and an anchor-poly-C primer. Although the technique has been used for characterization of the 5' end of other genes, the consistency and efficiency of the technique, as originally described, is variable depending, among other factors, on the target RNA. One possible explanation for the lack of consistency of the technique could be the need to eliminate all the oligoprimers before the tailing step to avoid tailing of the primer itself (J.F. Elliott, personal communication). To circumvent this problem, a modification of the technique has been recently reported (J. Kriangkum and J.F. Elliott, manuscript in preparation). Instead of removing the oligoprimer molecules before tailing with spermidine precipitation (which leads to a removal of around 95% of them), separation of the cDNA is performed by column chromatography. This cleaning step is more efficient for cleaning out oligoprimers.

Anchored PCR was performed using 25 μg of kidney total RNA from DBA/2J mice. Total RNA was reverse transcribed using oligo$(dT)_{18}$ as primer (1 μg; Boehringer Mannheim GmBH Canada Ltd.) in a 100 μl reaction containing 125 U of avian myeloblastosis virus reverse transcriptase (Boehringer Mannheim GmBH Canada Ltd.), 1 mM of each deoxynucleotide and 10 μl of $^{32}\alpha$P-dCTP (100 μCi), 6 U of placenta RNase inhibitor (RNasin$^{\bullet}$ ribonuclease inhibitor, Promega Corporation, Madison, WI), and PCR buffer (50mM KCl, 20mM Tris pH 8.4, 2.5mM $MgCl_2$, 0.1 mg/ml nuclease-free bovine serum albumin, and 1mM dithiothreitol (DTT)). The reaction was incubated at 42^0C for 90 minutes. After reverse transcription, the reaction was run through an A5M (Pharmacia) 4x300mm glass column which had been washed and packed with 0.1X TE. One drop fractions (25 μl) were collected and radioactivity was counted. The fractions (five to six) preceding the peak of radioactivity (usually appearing after 1 to 1.2 ml) were pooled and dried in a Speed-Vac. The pellet was resuspended in a 20 μl tailing reaction with Terminal Deoxynucleotidyl Transferase (15U; Gibco BRL), dGTP (0.5 mM), and tailing buffer (100 mM potassium cacodylate pH 7.2, 2 mM $CoCl_2$, 0.2 mM DTT). Tailing was performed at 37^0C for 30 minutes, after which the enzyme was inactivated at 70^{0}C for 5 minutes and the volume brought up to 100 μl with water. Five μl aliquots were taken and successively diluted at 1/5, 1/25, 1/125, 1/625, 1/3125, 1/15625, 1/78125, and 1/390625. These samples were then amplified in a 50 μl reaction containing Taq DNA polymerase (1 U) (Gibco BRL), sequence-specific sense and antisense oligonucleotides (1 μM), and PCR buffer to achieve the final concentrations mentioned above. The primers used for amplification were: antisense primer complementary to 18 bases located 38 bases downstream of the beginning of the TCR α C region (5'-TCCTGAGACCGAGGATCT- 3'), and sense primer containing an anchor with an internal NotI site plus 13 dCTP (5'-CTATCTAGAGAGCTCGCGGCCG(C)$_{13}$- 3'). PCR was performed at the following temperatures: denaturation at 92^{0}C for 1 minute, annealing at 50^{0}C for 1 minute, and extension at 72^{0}C for 1 minute. Amplification was carried for 35 cycles. To increase the amount of amplified products for cloning, a five μl aliquot of previously amplified samples were re-amplified for 35 cycles under the same conditions. Amplified products were run in a 2% agarose gel in TBE, and bands above the size of 50 nt long (to avoid cloning of primers and primer dimers) were cut from the gel, "gene-cleaned" with the GeneClean II Kit (Bio 101 Inc., La Jolla, CA). Amplified products were blunt ended in a 50 μl reaction containing deoxynucleotides (0.25 mM each), bovine serum albumin (500 ng/μl), DTT (0.1 mM), Tris-HCl (pH 7.5, 50 mM), $MgCl_2$ (10 mM), and Klenow fragment (4 U), at 37^{0}C for 15 minutes. The reaction was terminated at 72^{0}C for 5 minutes. Samples were then subjected to NotI digestion at 37^{0}C for 1 hr after addition of NaCl (100 mM) and NotI (30 U, Gibco BRL). After digestion, samples were "gene-cleaned" and eluted in 10 μl of water. The amplified products were ligated to pBluescript KS - (Stratagene) at molar ratio of 5:1 with T4 DNA ligase (2 U; Gibco BRL) and 5X T4 DNA ligase buffer (0.25 M Tris-HCl pH 7.6, 50 mM $MgCl_2$, 5 mM ATP, 5 mM DTT, 25% (w/v) polyethylene glycol-8000; Gibco BRL) at 16^0C for 2-18 hrs. Seven μl of the ligation reaction were used to transform TB1 E. coli competent cells. Transformed cells were plated in agar plates and incubated at 37^0C overnight. Clones were transferred to nylon membranes for screening.

Screening of plated bacteria for positive clones was performed using a single strand 43 nt probe complementary to the 5' region of the TCR α C region, upstream of the location of the antisense primer (5'-CGAGGATCTTTTAACTGGTA-CACAGCAGGTTCTGGGTTCTGGA-3'). Hybridization was carried out at 42^0C overnight in formamide, with no dextran sulfate (see details in the Material and Methods Section). Filters were washed once in 2X SSC, 0.1% SDS at room temperature, once in 1X SSC, 0.1% SDS at 55^0C and, if required, once with 0.2X SSC, 0.1% SDS at 55^0C. Although the probe overlaps nine bases with the antisense primer used for anchored PCR, under these washing conditions binding of the probe correlates with the presence of the whole TCR α C region upstream of the antisense primer.

Using anchored PCR, eight independent clones were obtained on the basis of hybridization with an internal TCR α C oligonucleotide 43 nt long. These clones were sequenced by the dideoxy method using a dsDNA Cycle Sequencing System

(Gibco BRL)(Figure 25). One of the clones (KTT106) contained the TCR α C region flanked by the TCR α C oligoprimer and the poly-C anchor. Five other clones (KPT19, KPT31, KPT40, KPT42, KT92) contained TCR α C region plus different lengths of a sequence, that after search in Gene Bank, showed to be Jα 11.2. Four of these five clones, extended bases beyond the end of Jα11.2. The upstream bases corresponded to germline DNA 5' to the Jα11.2 exon, with only a discrepancy in one G base, and included the heptamer RSS plus spacer. The other two clones (KPT17, KT105) contained two sequences that, at present time, have not been checked with GeneBank sequences and are of unknown origin.

Based on the predominance of independent clones containing the Jα11.2 region (71% of clones extending upstream of the TCR α C region) and on the finding of germline sequences on four of these clones, we believe that the truncated TCR α mRNA is the result of transcription starting upstream of Jα11.2 gene. The transcript is correctly spliced with the TCR α C region. In addition, preliminary data using RNase protection assay with one of the Jα11.2 clones using total RNA from kidney and spleen of scid and normal mice indicates that Jα11.2 is the predominant if not the exclusive component of the 5' end of the truncated TCR α mRNA. However, further screening is currently under way to exclude the possibility of heterogeneity of the 5' end of the truncated TCR α mRNA.

Thus far, analysis of the sequence upstream of the TCR α C region indicates that the transcript may be an open reading frame that may encode for a protein of about 30 kd. Further support for this hypothesis comes from the fact

```
gJα11.2 TGACTAAGAAAC ACT GTGG....................
Jα11.2                       GGG ACT GGA GGC TAT AAA GTG GTC TTT
KPT19   GGACTAAGAAAC ACT GTG GGG ACT GGA GGC TAT AAA GTG GTC TTT
KPT31            AAC ACT GTG GGG ACT GGA GGC TAT AAA GTG GTC TTT
KPT40            AAC AnT nTn GGG ACT GGA GGC TAT AAA GTn GTC TTT
KPT42                                                  nG GTC TTT
KPT92             AC ACT GTG GGG ACT GGA GGC TAT nAA GTn GTC TTT
KPT17                        AAn CAA AAG CnA GCn GCA GCC TGT nTG

Jα11.2           GGA AGT GGG ACT CGA TTG CTG GTA AGC CCT G**
KPT19            GGA AGT GGG ACT nGA TTG CTG GTA AGC CCT GAC ATC
KPT31            GGA AGT GGG ACT CGA TTG CTG GTA AGC CCT GAC ATC
KPT40            GGA AGT GGG ACT CGA TTG CTG GTA AGC CCT GAC ATC
KPT42            GGA AGT GGG ACT CGA TTG CTG GTA AGC CCT GAC ATC
KPT92            GGA AGT GGG ACT CGA TTG CTG GTA AGC CCT GAC ATC
KPT17            TnG ATG GAA AGC TAC AGC CCT GAA GTT CCG GAC ATC
KTT106                                                    AC ATC
KT105                                             TC TTT CCC ATC

KPT19            CAG AAC CCA GAA CCT GCT GTG TAC CAG TTA AAA GAT
KPT31            CAG AAC CCA GAA CCT GCT GTG TAC CAG TTA AAA GAT
KPT40            CAG AAC CCA GAA CCT GCT GTG TAC CAG TTA AAA GAT
KPT42            CAG AAC CCA GAA CCT GCT GTG TAC CAG TTA AAA GAT
KPT92            CAG AAC CCA GAA CCT GCT GTG TAC CAG TTA AAA GAT
KPT17            CAG AAC CCA GAA CCT GCT GTG TAC CAG TTA AAA GAT
KTT106           CAG AAC CCA GAA CCT GCT GTG TAC CAG TTA AAA GAT
KT105            CAG AAC CCA GAA CCT GCT GTG TAC CAG TTA AAA GAT

KPT19            CCT CGG TCT CAG GA
KPT31            CCT CGG TCT CAG GA
KPT40            CCT CGG TCT CAG GA
KPT42            CCT CGG TCT CAG GA
KPT92            CCT CGG TCT CAG GA
KPT17            CCT CGG TCT CAG GA
KTT106           CCT CGG TCT CAG GA
KT105            CCT CGG TCT CAG GA
```

Figure 25.- Sequence of the 5' end of the truncated TCR α mRNA. cDNA obtained after reverse transcription of total kidney RNA was amplified by anchored PCR. Amplified products were then cloned and sequenced by the dideoxy method. Sequence of the germline DNA upstream of Jα11.2 (gJα11.2) and of the coding region of Jα11.2 (Jα11.2) are shown for comparison. ** show the begining of the Cα region.

that the germline DNA sequence upstream of the Jα11.2 gene shows the presence of an ATG codon 46 bases upstream in frame with the sequence obtained thus far. Confirmation of the open reading frame may come after cloning of longer cDNAs.